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Ph.D. Dissertation
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Integrated –omics approaches to explore tomato interaction with the leafminer *Tuta absoluta*

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ABSTRACT

Tuta absoluta is one of the most destructive pest affecting tomato crops, causing considerable field and greenhouse yield losses. Despite its economic importance, little is known about the molecular basis of the interaction between this leafminer and tomato plants. To investigate the tomato response to *T. absoluta* challenge , a multi-omic approach was carried out. Tolerant and Susceptible cultivated tomato genotypes as well as the derived F1 hybrid were employed to shed light on the plant response to the herbivore feeding. A RNA-Seq experiment was performed to analyze the transcriptional reprogramming of three tomato genotypes infested by *T. absoluta*. Metabolome fingerprinting analysis was carried out both to analyze differences in metabolites production among infested and not infested genotypes and to validate gene expression findings. Furthermore, a structural genomic-based analysis allowed us to identify polymorphisms such as SNPs and InDels affecting genes putatively involved in tomato-*T. absoluta* interaction. In the Tolerant genotype, the reprogramming driven by both direct and indirect defenses is based on an antixenosis mechanism. This is characterized by the lower utilization of the host by the herbivore due to chemicals, physical and morphological barriers. The RNA-Seq gene expression analysis allowed us to assess an active recognition of the insect that leads to a signaling cascade mediated by the systemin/jasmonic acid complex and, subsequently, the activation of genes involved in the growth of trichomes (physical barriers) together with the activation of genes coding for production of volatile terpenes and phenylpropanoids. A direct defense has been well elucidated by the metabolome analysis, revealing an involvement of compounds such as chlorogenic and neo-chlorogenic acids, GABA and, pyridinc alkaloid trigonelline. The susceptible line demonstrates to be less capable of deploying with the defense arsenal. The key genes identified in JA, terpenes and phenylpropanoids pathways resulted down-regulated and affected by deleterious variants that could lead to important changes in the final protein synthesis. The F1 derived from the cross between the Tolerant and Susceptible lines expresses, even if at less extent, the key genes identified in the tolerant line, and is affected by the same structural polymorphisms. Results obtained in this thesis suggest that the tolerance to the leafminer *T. absoluta* is modulated both by structural variations and by the expression regulation of such genes . The findings gathered in this study could be very useful for better direct future tomato breeding for *T. absoluta* tolerance.

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1. INTRODUCTION

1.1 Tomato history and importance

The tomato (*Solanum lycopersicum)* belongs to *Solanaceae* nightshade family and it is one of the most economically important vegetable worldwide. In 2012, tomato production was valued at 58 billion dollars and such crop was the eighth most valuable agricultural product in the world; Italy is the main European tomato producer and in 2012 resulted to be the 7th highest world producer. *Solanum lycopersicum* originated from the Andean part of South America, including regions of Chile, Boliva, Ecuador, Colombia and Peru. It was firstly introduced in Europe by Spanish explorers at the beginning of 16th century as a botanical curiosity and its potential as a foodstuff was hidden behind the suspicion of the presence of alkaloids in the fruits. It was only in the 17th century that this species started to be appreciated as an edible fruit and then its cultivation diffused all through the world. In Europe, the tomato was rapidly spread in the Mediterranean countries, including Spain and Italy from where the species was reintroduced in North America in the 18th century (Jenkins, 1948). Due to its success in cultivation, *S. lycopersicum* found a secondary centre for diversification in the above mentioned countries (Zhou et al., 2015). Especially in Italy, seed selection made by farmers and growers produced local adapted germplasm. At this purpose, within the EU, there are several areas that grow tomato varieties with Protected Geographical Status, among them San Marzano dell'Agro Nocerino - Sarnese (PDO), in Southern Italy, or Pomodorino del Piennolo del Vesuvio (PDO), in Mount Vesuvius area, in Naples. These traditional tomato accessions are believed to have valuable traits in terms of agro-ecological adaptation, consumer preference and sensory quality (Ercolano et al., 2008). Tomatoes are one of the low-calories vegetables (18 calories per 100 g) with a very low fat contents and zero cholesterol levels. Nonetheless, they are an excellent sources of antioxidants, dietary fiber, minerals and vitamins. The decoding of the Heinz 1706 tomato reference genome by the multi-national Tomato Genome Consortium (2012), allowed to better understand the genetic basis of agronomic traits facilitating the development of new cultivars.

Tomato crop is susceptible to a whole plethora of abiotic and biotic stress. Among the latter, in tomato field of Mediterranean area virus and insect pests produces the most threatening and yield-lossing damages. Chemical control to combat such threats is often too expensive for growers and, in some cases, ineffective. Moreover, the use of pesticides has been reduced due to environmental and consumer constraints. Hence, the development of resistant cultivars results to be one of the most important research goals for promoting a sustainable agriculture. Tomato was extensively used as a

model plant for resistance studies and important progresses has been obtained through both genetic and biotechnological approaches (Ercolano et al., 2012). In the last years, several resistant cultivars to diseases have been released. However other treats, that can totally destroy tomato crops, such as *Tuta absoluta*, have emerged.

1.2 *Tuta absoluta* (Meyerick)

The tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: *Gelechiidae*) is one of the most devastating and harmful pests of Solanaceous crops. In its native area (South America), as well as in newly invaded regions, the moth preferentially attacks tomato under both field and greenhouse conditions. In 2004, *T. absoluta* was added by the European and Mediterranean Plant Protection Organization (EPPO) to the A1 List of pests recommended for regulation (pests absent from the EPPO region), and in 2009 was transferred to the A2 list (pests locally present in the EPPO region), 3 years after its arrival in Spain. During 2006–2012, the pest spread rapidly through-out the Mediterranean basin (Desneux et al., 2010; Tropea Garzia et al., 2012). Since the time of its initial detection, the pest has caused serious damages to tomato in invaded areas, and it is currently considered as a key agricultural threat to European and North African tomato production (Viggiani et al., 2009). Consequently, the ongoing invasion of *T. absoluta* has amplified applied research to undertake studies on many aspects of its biology and ecology (Zappalà et al., 2012; Baetan et al., 2015). *T. absoluta* was firstly described in 1917 by Meyrick as *Phthorimaea absoluta*, from a single male collected in Peruvian Andes. It was placed in the genus *Tuta*as *T. absoluta* by Povolny many years later (1994), after having been previously reported as *Gnorimoschema absoluta* (Clarke), *Scrobipalpula absoluta* (Povolny) and *Scrobipalpuloides absoluta* (Provenly) (Desneux et al., 2010). The moth is a multivoltine pest that shows high reproductive potential and short life cycle (Pereyra & Sanchez, 2006). Females lay eggs on leaves and stems, the young larvae bores and develops inside the plant, continuously searching for new feeding locations and pupation occurs mainly in the soil. If food is available and climatic conditions are favorable, larvae feed almost continuously and generally do not enter diapause. In the Mediterranean basin, *T. absoluta* infests tomato as well as other Solanaceous crops (eggplant, sweet pepper, potato and tobacco). All the epigeal tomato plant parts are suitable for the moth development. Larval feeding activity reduces the plants photosynthetically active surface and, consequently, growth and yields (Bogorni et al., 2003). The plant can be attacked in all its developmental stages and severe injuries to seedlings can occur, leading to the death of young plants when larvae develop inside the main stem (Pereyra & Sanchez, 2006). In addition, multiple wounds on the tissues caused by larvae make the plants more vulnerable to secondary diseases, especially those caused by bacteria, which can actively penetrate

the damaged tissues. Control of *T. absoluta* is a worldwide necessity but the efficacy of foliar insecticides is inconsistent, as it may require many applications with undesirable effects (residues, damage to natural enemies, resistance to chemicals, etc). Eco-sustainable control methods and integrated pest management (IPM) programs are needed for contrast such treat. In this framework a key role could be played by biological control agents and by the development of new tomato varieties resistant to the leafminer. Wild species resistant to *T. absoluta* such as *S.pennelli* and *S. habrochaites* have been identified. A number of studies have enlightened that different compounds such as Zingiberene (de Azevedo et al., 2003), Acylsugars (de Resende et al., 2006) and 2-Tridecanone (Maluf et al., 1997) are able to confer resistance to *T. absoluta.*

1.3 Plant-Insect interactions

Phytophagous insects represent a huge problem in global crop cultivation causing yield reductions and considerable costs in control measures. Approximately 10.000 different insect species are considered worldwide crop pests causing annual production losses estimated at $400 billion in 2004 (Pimentel et al., 2005). Huge resources are currently directed at the control of insect pests through the application of synthetic insecticides, which have been proven highly effective in the past, but a number of factors have led to a decrease in the efficacy of insecticides over time. Among those factors, the switch to crop varieties that have inherently lower insect resistance, the destruction of natural enemies of insect pests through a lack of insecticide specificity and the development of insecticide resistance (Fenton et al., 2010). Integrated pest management strategies seek to control pests through the selection of resistant cultivars, the use of appropriate crop rotations, the encouragement of insect predators. Key to such strategies is the development of crop varieties that exhibit specific resistance and/or tolerance traits allowing maintenance of crop yield. This particular aspect pushed the boundaries of research, making considerable progresses towards our understanding of the complexity of plant responses to insect infestation and the molecular bases of resistance and tolerance traits. *Resistance* and *Tolerance* are two different plant defense strategies against herbivores: the first can be defined as the ability of a plant to avoid or limit damage from herbivores by reducing herbivore preference or performance due to the presence of constitutive responses; tolerance is the ability of a plant to maintain fitness following herbivores feeding (Nùñez–Farfàn et al., 2007). Research about plant-herbivores interaction has revealed, during the last 30 years, the plasticity of plants responses to the insect attacks. These have been categorized into: (1) *indirect defence* in which chemical cues are released to recruit predators and parasitoids to control herbivores; (2) *direct defence* through the production of toxic compounds; and (3) *tolerance* where herbivory results in little or no reduction in plant fitness (Baldwin & Preston, 1999; Turlings

& Ton, 2006). A common starting point of all these three categories is related to the recognition of attackers, the induction of signal transduction pathways and the induction of biosynthetic pathways leading to changes in plant phenotype. Successful defense depends on the ability of the plant to recognize an attacking 'enemy' as early as possible. The recognition of herbivores depends also on its feeding habits and it is mediated by plant receptors that initiate a cascade of responses, including changes in plasma membrane potential and activation of networks of kinases and phytohormones (Maffei et al., 2007). Insects employ various feeding strategies to obtain nutrients from all plant parts. Although all phytophagous insects inflict mechanical damage on plant tissues, quantity and quality of injury vary greatly, depending on the feeding tactic. Approximately two-thirds of all known herbivorous insect species are leaf-eating beetles (Coleoptera) or caterpillars (Lepidoptera) that cause damage with mouthparts evolved for chewing, snipping, or tearing (Schoonhoven et al., 1998). Piercing-sucking herbivores such as thrips and spider mites use tube-like structures to suck the liquid content from lacerated cells, whereas leafminers develop and feed on soft tissue between epidermal cell layers. Responses to these kind of insects depend also on feeding habits, although, the central role of phytohormones as regulatory mechanisms underpinning plant defense responses to insect herbivore attack have been assessed (Erb et al., 2012). Much less is known about signaling pathways involved in resistance against insects of other feeding guilds, such as leafminers, stem borers, leaf folders, and gall-inducing herbivores. Among phytohormones, jasmonic acid's (JA) pivotal role in plant development and resistance to biotic stresses has been well documented (Browse, 2009) and, as part of the plant immune system, JA confers resistance to necrotrophic pathogens (Kunkel & Brooks, 2002; Vijayan et al., 1998). Many studies have demonstrated that JA is the most important hormone that controls plant defense against herbivores. Drastically decreased resistance was observed in plants with impaired biosynthesis or perception of JA; the compromised resistance is usually associated with the highly attenuated accumulation of defensive compounds in these plants (Halitschke & Baldwin, 2003; Wang et al., 2008). Moreover, transcriptome analyses using microarrays indicated that a large portion of wounding- and herbivory-induced responses are mediated through the JA pathway (Reymond et al., 2000; Reymond et al., 2004). The broad-spectrum defense responses, hence, can be achieved via JA-independent processes and spatio-temporal changes of JA-modulating hormones, including ethylene, salicylic acid, abscisic acid, auxin, cytokinins, brassinosteroids and gibberellins (Erb et al., 2012).

1.4 ‘-Omics’approaches

The recent advances in the generation of high-throughput data such as whole-genome sequencing, RNA-seq analysis and metabolome profiling allows to accelerate discovery. “High-throughput”refers to a technology in which a large (or even exhaustive) number of measurements can be taken in a fairly short time period. "Ome" and "-omics" are suffixes that are derived from genome (the whole collection of an organism's DNA, as coined by Hans Winkler, as a combination of "gene" and "chromosome") and genomics (the study of the genome). Scientists like to referto these to any large-scale system, such as the collection of proteins in a cell or tissue (the proteome), the collection of metabolites (the metabolome), and the collection of RNA that's been transcribed from genes (the transcriptome). Typical –omics approaches include genomic, proteomic, transcriptomic, metabolomic, phenomic, interactomic, ionomic, etc approaches. Each of these -omics technique on its own can provide useful and novel information about biological process, but data from several approaches may also be integrated together to facilitate the identification of genetic traits underlying a given phenotype. Utilizing available high-throughput multiomics data along with robust bioinformatics and data mining tools, scientists can explore relevant correlations and construct models describing different biological processes (Fukushima et al., 2009). Nowadays, plant biologists have been using high-throughput -omics techniques extensively in their research also in plant–insect interactions (Mochida & Shinozaki, 2011). Knowledge generated from such integrative plant–insect interaction studies can also be used for integrated pest management (Ahuja et al., 2011). Among all the above mentioned –omics approaches, three are proposed for studying plant-herbivores interactions and described as follows.

1.4.1 Genome-based studies

The interactions between plants and insect herbivores are among the most important processes in terrestrial ecosystems (Crawley, 1989; Huntly, 1991; Schmitz, 2008) and are believed to contribute fundamentally to the evolutionary diversification of both plants and insects (Mitter et al., 1991; Marquis, 1992; Price et al., 2011). Genetic and genomic variation among individual plants in their susceptibility to herbivore attack serves as the basis for the evolution of resistance and tolerance in natural plant populations through natural selection and for crop improvement through selective breeding. Identifying genomic variation is a crucial step for unveiling the relationships between genotype and phenotype and can yield important insights into plant’s biology. Moreover, next generation sequencing (NGS) technologies have facilitated the identification of genetic variations. Among them, the Single Nucleotide Polymorphisms (SNPs) have been extensively used for genetic

linkage analysis, population genetics, genetic resource characterization in order to find important agronomic traits for crop breeding (Causse et al., 2013; Ercolano et al., 2014; Sacco et al., 2015). Characterization of the SNP densities within a genome and between individuals has led to the identification of numerous genes, genetic regions, and distinguishing genetic features related to important agronomic traits. Furthermore, the availability of different plant genomes, has greatly facilitated this endeavor, providing the study of nucleotide diversity in a multitude of plant species. Millions of polymorphisms have been discovered in Arabidopsis (Gan et al., 2011), rice (Xu et al., 2012), soybean (Lam et al., 2010), maize (Lai et al., 2010) and tomato (Blanca et al., 2012). In plants, variants discovery can be performed either from RNA-Seq experiments (Choi et al., 2007) or whole genome re-sequencing. RNA-Seq is arguably a more popular application because it costs less than genome sequencing and has the ability to address a multitude of different questions, such as the quantification of gene expression levels, detection of alternative splicing, allele-specific expression, etc. The alignment of short reads to a reference sequence allows the discovery of different types of sequence variations, including single nucleotide polymorphisms (SNPs), short insertion/deletions (InDels) and copy number variants (CNVs). The accuracy of read alignment (and the calling of a variant) can vary significantly with the efficiency of base-calling and the presence of InDels or erroneous base calls generated during sequencing. Moreover, the unique architecture of plant genomes often means that a large proportion of short reads will align to several possible genomic locations. In this framework, the development of bioinformatic tools has reached a fundamental role.

1.4.2 Transcriptomic-based studies

One of the globally measurable events of plant responses to herbivores is the changes in the levels of gene expression. Since the development of a first microarray chip detecting 45 transcripts of *Arabidopsis thaliana* (Schena et al., 1995), the field of transcriptomics has gone through revolutionary changes and is currently considered as a major –omic technique for studying plant responses to insect attack (Thompson & Goggin, 2006). In addition, rapidly developing cost-effective next-generation sequencing NGS-based technologies may now be applied to carry out transcriptomic analysis. Several high-throughput platforms can generate millions of sequences in parallel within a short span of time are available (Egan et al., 2012; Mardis, 2013). Briefly, a typical RNA-Seq experiment starts with mRNA extraction that is subsequently converted into cDNA to form a ‘library’. By sequencing the millions of DNA fragments in the library (known as ‘reads’) with next-generation sequencing platforms, an accurate measure of the relative abundance of each transcript and splice variants can be obtained. Then, obtained sequence reads will be aligned to a

reference genome and/or transcriptome to translate next-generation sequencing output into a biological information; a wide array of bioinformatics tools have been developed to process all the individual steps and to provide useful information on gene expression levels. Since transcriptional reprogramming typically underlies plant defense responses to herbivores, many transcriptomic analyses of responses to insect herbivores have been conducted with RNA sequencing using several plant species. Tzin and colleagues (2015) assessed the role of specific metabolic pathways in aphid-infested maize plants. Gene expression changes revealed a predominant effect of salicylic acid regulation and a prolonged induction of oxylipins, not necessarily related to Jasmonic Acid pathway. Successful use of NGS technology to develop transcriptomic and genomic resources, including expressed genes and molecular markers for a non-model invasive aphid species *Aphis glycines,* was demonstrated by Bai et al. (2010).

1.4.3 Metabolomics

Metabolomics analyses can provide valuable information about plant defense to insects on its own (e.g. by identifying new interesting compounds, by looking at local and systemic changes) but may also be combined with other -omics approaches in an attempt to link phenotype and genotype (Macel et al., 2010). One of the most universally used metabolomic approaches comprises nuclear magnetic resonance spectroscopy (NMR). NMR spectroscopy measures the resonances of magnetic nuclei such as ^{1}H, ^{13}C and ^{15}N that interact with an external magnetic field (Hatada & Kitayama, 2004). It offers non-invasive structural analysis of metabolites in crude extracts, cell suspensions, intact tissues or whole organisms. NMR in plant metabolomics has a wide range of applications. Plants produce an immense number of secondary compounds to interact with beneficial or harmful organisms. These compounds are mainly secondary metabolites with no major involvement in the normal growth, development or reproduction of the plant. Such compounds can act as signalling molecules (Zebelo & Maffei, 2012) or direct defence chemicals, and include alkaloids, terpenoids, cyanogenic glycosides, glucosinolates and phenolics (Bennett & Wallsgrove, 1994). Along with secondary metabolism, a plant's primary metabolism is also differentially affected during an insect or a pathogen attack (Barah et al., 2013). Studying the differential regulation of primary or secondary metabolites during plant–insect interaction has been in practice from long time (Weckwerth & Kahl, 2013). Metabolite changes induced by herbivore in leaves, roots, root exudates and vascular sap from stems were revealed in monocot and dicot plant species; maize plant submitted to metabolite profiling after leaf infestation with *Spodoptera littoralis* revealed changed concentration levels of more than 30 compounds upon insect attack (Marti et al., 2013). Quantitative and qualitative differences in metabolite changes among tissues and between insects

were assessed in undamaged leaf, apex, stem and root tissue from tomato after infestation with one of two insect herbivores (*Manduca sexta* or *Helicoverpa zea*) (Steinbrenner et al., 2011). Leiss et al. (2009, b) developed an eco-metabolomic approach, based on NMR, to identify candidate compounds for constitutive host plant resistance to western flower thrips (*Frankliniella occidentalis*). The effect of herbivores on plants has also been studied with NMR spectroscopy for chewing insects such as the caterpillars *Plutella xylostella* and *Spodoptora exigua* in *Brassica rapa* (Widarto et al. 2006).

1.5 Aims of the project

A multi-omic approach was carried out in this study in order to investigate the interaction between tomato and the leafminer *T. absoluta*. Tolerant and Susceptible cultivated tomato genotypes as well as the derived F1 hybrid were employed to shed light on the plant response to the herbivore feeding. In this framework, a RNA-Seq experiment was performed to analyze the extent transcriptional reprogramming of three tomato genotypes infested by the leafminer *T. absoluta*. Metabolome fingerprinting analysis was carried out both to analyze differences in metabolites production among infested and not infested genotypes and to validate gene expression findings. Furthermore, a structural genomic-based study was conducted to assess if differences in response to the herbivore among tolerant and susceptible genotypes are driven by polymorphisms affecting genes putatively involved in the response to *T. absoluta*.

2. MATERIALS AND METHODS

2.1 Plant material

Three tomato (*Solanum lycopersicum*) genotypes were provided by FARAO seed company (Sarno, Italy). A putatively tolerant/partial resistant cherry type tomato BR221 (from now on named as 'T') and a susceptible variety, PS650 (from now on named as 'S') were used in the experiment. These two genotypes were furthermore used as parental lines (Tolerant x Susceptible) to obtain an F1 hybrid CS823 (from now on named as 'F1'), also used in the experiment.

2.2 Field trials and samples collection

A special tunnel (90 x 60 cm) consisting of two nylon cages divided with a septum was build up in a greenhouse to perform the infestation trials on 20 cm high tomato plants. Each cage contained the genotypes under study in a randomized complete block design consisting of 20 plants/replica for each genotype for each condition. A total of 120 plants were used for the experiment. Since the cages were divided with a septum, it was possible to collocate on a side the plants to be exposed to the leafminer and on the other side control tomato plants. Plants were artificially infested with 320 *T.absoluta* adults and remained in the infestation cage for at least 45 days, when an overall plant damage was visually assessed. At this time point, leaves with and without mines from each plant were singly collected and immediately frozen in liquid nitrogen.

2.3 TRANSCRIPTOMICS

2.3.1 RNA isolation and evaluation

T, S and F1 collected leaves from three-four tomato plants of each replica were furthermore pooled together in six pools of samples for diminishing sampling errors and for obtaining a higher quantity of extracted RNA. The single sample pools were stored at -80°C. Total RNA purification was performed on 100 mg of frozen tomato leaves without *T. absoluta* visual damages using Spectrum™Plant Total RNA Kit (Sigma Aldrich), according to manufacturer's protocol. A treatment with On-ColoumnDNaseI Digestion Set (Sigma Aldrich) was carried out on total RNA to remove genomic DNA contaminations. RNA purified samples were quantified by NanoDrop ND-1000 Spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA) and its integrity was confirmed using the Bioanalyzer (Agilent Technologies). RNA integrity was furthermore checked

by horizontal electrophoresis on a 1% (w/v) agarose gel with GelRed Nucleic Acid Stain 10,000X (Biothium) by UV light (UV Gel Doc BIORAD). Samples with required standards of quality were subjected to sequencing at the LabMedMolGe (Laboratory of Molecular Medicine and Genomics Department of Medicine and Surgery, University of Salerno).

2.3.2 RNA Sequencing and mapping

Total purified RNA was converted to cDNA libraries and sequenced on Illumina HiSeq1500 platform at the LabMedMolGe (Laboratory of Molecular Medicine and Genomics Department of Medicine and Surgery, University of Salerno). The protocol included strand-specific library preparation followed by paired-end 100 base pair (bp) sequencing. To evaluate the quality of the sequences generated the FastQC software was used (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). A quality check was performed on the raw sequencing data in order to obtain high quality reads, with a minimum length of 25 bp and a quality score of 35. The total number of reads, before and after the trimming, is presented in Table S1 (Supplementary Materials). RNA-Seq analysis was performed in three different steps: raw reads processing, data statistical analysis and functional annotation of the identified loci. High quality reads were aligned against the *Solanum lycopersicum* reference genome sequence (S_lycopersicum_chromosomes.2.50) with TopHat (version 2.0.11). Uniquely mapping reads were used as input for FeatureCounts (Subread package, version 1.4.4) together with the ITAG2.4 annotation file to calculate gene expression values (read counts). The overall experiment was evaluated on the basis of the similarity between replicates by a PCA analysis as well as by the algorithm SERE that calculates similarity scores among samples assuming a binomial distribution of the read counts. The HTSFilter package, which implements a filtering procedure for replicated transcriptome sequencing data based on a Jaccard similarity index, was used for removing genes of each experiment with a very low read count or those that were too variable among the replicates of each experimental condition. The "Trimmed Means of M-values"(TMM) normalization filter was applied to the different experimental conditions in order to identify and remove genes that appear to generate an uninformative signal. Differentially expressed genes (DEGs) identification was performed using the DESeq2 package considering all the genes passing the HTSFilter step.

2.3.3 Transcriptome data analysis

The annotation of biological information related to the identified differential expressed loci was performed using ITAG2.4 protein functional annotation file. *AgriGO*

(http://bioinfo.cau.edu.cn/agriGO/) web tool was used to carry out a Gene Ontology Enrichment Analysis, using an FDR cut-off value of 0.05. DEGs assignment to specific metabolic pathways was performed using *MapMan* 3.0.0 tool (Usadel et al., 2009) and *Plant MetGenMap* (http://bioinfo.bti.cornell.edu/cgi-bin/MetGenMAP/home.cgi) visualization and analysis package. Input files for the above mentioned mapping tools were prepared taking in account the log2foldchange and p-value adjusted values of DEGs. The Sol Genomics (www.solgenomics.net) database was useful to find more information on annotated genes, while SolCyc (www.solcyc.solgenomics.net) was used to obtain detailed information on pathways and biochemical reactions involved in the tomato-*T. absoluta* interaction.

2.4 METABOLOMICS

2.4.1 Extraction procedure

Samples in triplicates were dried and then dissolved in 5 mL of CH_2Cl_2/MeOH/H_2O in ratio of 2:1:1. After sonication (1 min), each mixture was centrifuged at 3000 rpm for 30 minutes at room temperature and then the aqueous and the organic fractions were accurately separated. The extraction was repeated twice. The solvent of each extract was evaporated to dryness under vacuum (Rotavapor R-114, Büchi, Switzerland) and dry residues were kept at 4°C until NMR analysis. Samples were prepared as described above and analyzed using NMR platform, and the intensity of selected signals was measured. The obtained values showed a very good repeatability, with coefficient of variation among replicates < 2.5% for all signals.

2.4.2 NMR Experiments

Dried aqueous fractions were diluted in 600 µl of deuterium oxide (99,8% D_2O) while dried organic fractions dissolved in 600 µl of chloroform-d (99,8% $CDCl_3$) and transferred into a 5 mm NMR tube. Tetramethylsilane (TMS) was used as an internal standard. The NMR spectra were recorded at 298 K on a Varian Unity Inova spectrometer operating at 400 MHz. For each sample 200 transients were recorded using a spectral width of 12 ppm on 32K data points and relaxation delay = 0.01 sec. Chemical shifts were referred to TMS signal (δ0.00 ppm). All spectra were processed using iNMR program (www.inmr.net), phased and baseline corrected. Quantification was performed by signal integration relative to the internal standard, TMS. The region of the solvent peaks was excluded from the analysis. All spectra were manually phased and baseline corrected.

2.4.3 Multivariate Data Analysis

Multivariate analyses were applied to ^{1}H NMR spectral data.^{1}H NMR spectra were preliminarily normalized and reduced to integrated regions of equal widths (bins = 0.01 ppm), corresponding to 0 –10 ppm and subsequently reduced to ASCII files using iNMR. Matrices were submitted to Principal Component Analysis (PCA) ordination using the STATISTICA 7 Software (StatSoft Inc., Tulsa, Oklahoma, USA).

2.5 GENOMICS

2.5.1 Variant calling and annotation

The SUPERW (Simply Unified Pair-End Read Workflow) pipeline (http://sourceforge.net/projects/superw/) was used to carry out the Variant calling analysis. For the filtering and mapping steps raw reads produced by RNA-Seq experiment were used as input and automatically filtered in order to create a new high quality subset of reads suitable for mapping analyses. RNA sequencing experiment was performed on T, S and F1 infested and non-infested triplicate samples. The protocol included strand-specific library preparation followed by paired-end 100bp sequencing. A quality check was performed on the raw sequencing data in order to obtain high quality reads, with a minimum length of 25 bp and a quality score of 35. Cleaned reads were then mapped against the *Solanum lycopersicum* reference genome (version 2.5) with BWA (Li & Durbin, 2009) using the bwa-aln algorithm. Parameters used for the mapping were insert size of 500 bp and mapping quality of PHRED >10. Mapped files were then filtered for PCR duplicates, compressed in bam files, sorted and indexed (Li et al., 2009) creating as output a bam file. The calling of small variations (SNPs and InDels) was performed with SAMtools (Li et al., 2009) through a double calling step using the bam output files and the reference genome. A first run of SAMtools performed a multiple pileup (Mpileup) in which all the samples are used together to perform the SNPs and InDels calling, while a second run is used to call small variations independently for each sample. Both of the outputs from these analysis were then merged together for the final result. Variant effect analysis of SNPs and InDels, and their positions in the genome was predicted using SnpEff (http://snpeff.sourceforge.net/) starting from the .vcf files obtained from the SUPERW pipeline. A tomato reference database, including the tomato reference genome and the genome annotation (SolGenomics Network, ITAG2.5), was created and used to categorize the effects of the allelic variants. Effects were classified by impact (High, Moderate, Low and

Modifier) and effect (synonymous or non-synonymous amino acid replacement, start codon gain or loss, stop codon gain or loss or frame shifts, etc). For each chromosome, distribution of variants for a window of 1MB has been determined and graphically depicted. Based on MapMan gene categorization of DEGs, a functional classification of the genes with interesting allelic variants for each genotype and impact category was performed.

2.5.2 Cluster analysis of DEGs

Distribution and clusterization of DEGs along chromosomes of T, S and F1 tomato genotypes was performed with REEF software (http://telethon.bio.unipd.it/bioinfo/reef/).This tool aimed at identifying genomic regions enriched in specific features, such as a class or group of genes homogeneous for expression and/or functional characteristics. The method for the calculation of local feature enrichment uses test statistic based on the Hypergeometric Distribution applied genome-wide by using a sliding window approach and adopting the False Discovery Rate for controlling multiplicity. Parameters used for this analysis were set as 400kb for the sliding window size, 200kb for the shift between adjacent windows and a minimum number of 5 genes in each window. Visualization of REEF outputs was performed with ggbio R package (Release 3.2) (Yin et al., 2012).

3. RESULTS

3.1 TRANSCRIPTOMICS

3.1.1 Transcriptome sequencing

Six RNA-Seqpaired-end 100bp libraries from three tomato (*Solanum lycopersicum*) genotypes (BR221/ PS650/ CS823-F1 hybrid) were sequenced using Illumina technology. In order to ensure a high quality and reliability of the analysis a minimum sequence length of 25 bp and a sequence quality score of 35 was established. An average of 24.3 million of fragments per sample was obtained after the filtering process (Supplementary materials: Table S1). BR221 (T), PS650 (S) and CS823-F1 hybrid (F1) reads were then mapped to the tomato reference genome (S_lycopersicum_chromosomes.2.50). The resulting alignment files were used, together with the ITAG2.4 annotation file, to calculate gene expression values (read counts). Only uniquely mapping reads were used for read counting. Approximatively 19.000, 21.000 and 21.000 loci, respectively for T, S and F1 hybrid (Supplementary materials: Figure S1, panel a-b-c), were identified and retained for further analysis.

3.1.2 Differentially expressed genes identification

To assess gene expression changes after the *T. absoluta* disturbance, RNA-seq data of T, S and F1 hybrid infested and non-infested tomato genotypes were compared. The differentially expressed genes(DEGs) were identified computing for each genotype data obtained from infested against non-infested samples.

- Tolerant infested VS Tolerant non-infested (T_i vs T_ni);
- Susceptible infested VS Susceptible non-infested (S_i vs S_ni);
- F1 infested VS F1 non-infested (F1_i vs F1_ni).

Differentially expressed genes profiles are showed in Figure 1. The Tolerant genotype (T_i vs T_ni) showed the major gene expression changes after a *T. absoluta* challenge (8612 DEGs). As for the the Susceptible genotype (S_i vs S_ni) 4436 total DEGs were obtained while, for the F1 hybrid (F1_i vs F1_ni), 1301 DEGs were detected. Interesting differences can be observed also for the gene expression levels, since all the three genotypes showed more up-regulated than down-regulated genes. In particular the T showed a total number of 4482 up-regulated and 4130 down-

regulated transcripts; S showed out of the total number, 2566 up-regulated and 1870 down-regulated DEGs; the F1 hybrid resulted of 934 up-regulated genes and 367 down-regulated in the *infested vs not infested* condition. The experimental design also allowed us to perform a comparison among genotypes. At this purpose, DEGs obtained in all genotypes were crossed in a Venn diagram, in order to distinguish the unique and the common DEGs among genotypes in the two experimental conditions (Fig. 2). Even in this case the tolerant genotype evidenced the highest number of specifically expressed genes, 4406 was evidenced in T genotype, while 674 DEGs were unique for S and 128 for F1. Moreover, it's interesting to note how the F1 hybrid shares more genes with the T genotype (489) than the S genotype (49).

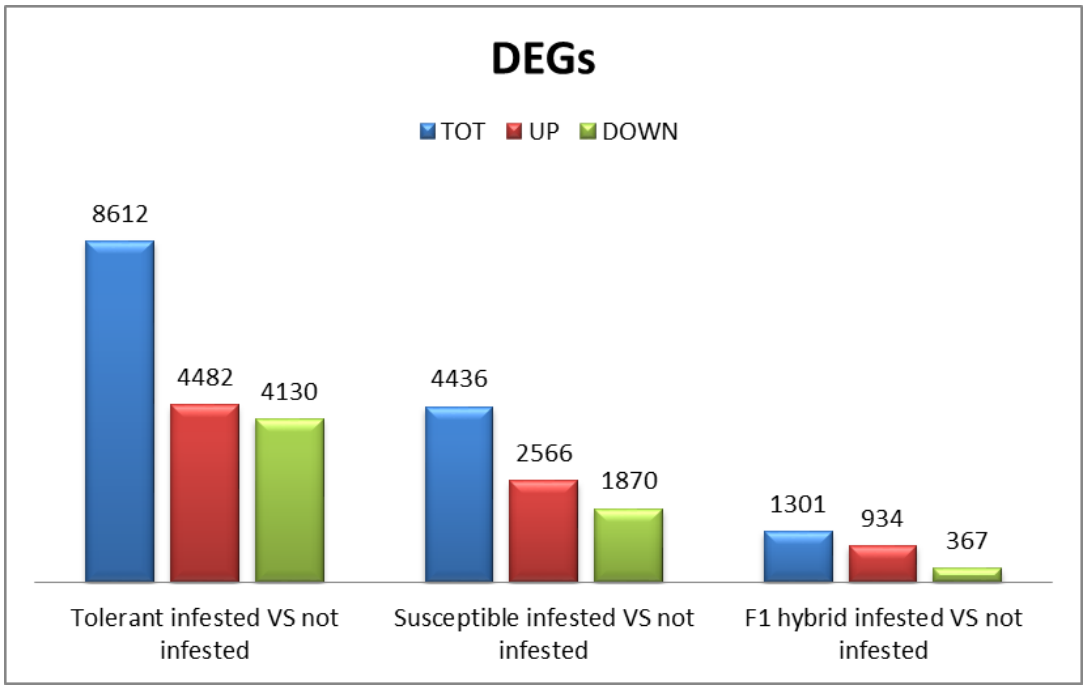

Figure 1. Comparison of differential gene expression patterns among the three analyzed genotypes. Total number, UP-regulated and DOWN- regulated differentially expressed genes (DEGs) are presented as bars.

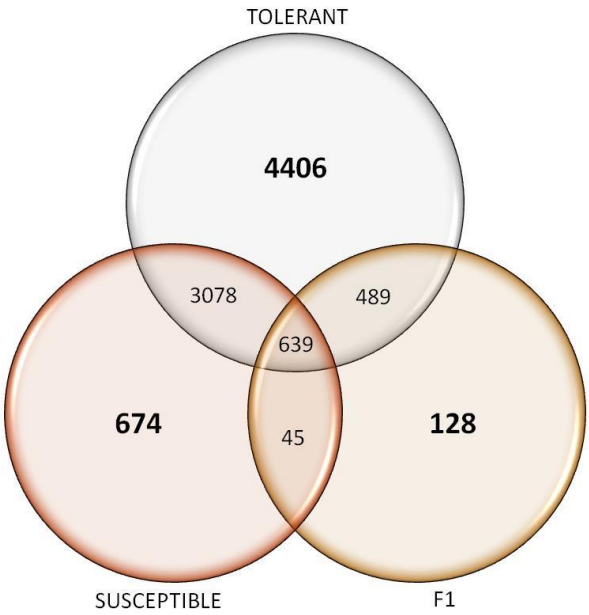

Figure 2. Venn Diagram representing the Unique and Common DEGs among the three genotypes tested in *infested vs non-infested* condition.

3.1.3 Functional annotation

In order to get information on main pathways challenged during the tomato responses to *T. absoluta*, a functional annotation of the differentially expressed transcripts has been carried out. The results obtained by a Gene Ontology enrichment analysis and a DEGs metabolic mapping, using both *MapMan* and *PlantMetGen Map* tools, were integrated to provide an overview of tomato-*T. absoluta* interaction.

3.1.3.1 Gene Ontology terms enrichment analysis

A GO (Gene Ontology) term annotation analysis of all identified transcripts was performed to identify over-represented gene classes in the three tomato genotypes (T; S; F1) in i vs ni condition. This analysis was carried out on both unique and common genes of the three genotypes in order to obtain the 'genotype-specific' and the 'common' GO terms. Gene Ontology analysis performed on unique DEGs datasets allowed us to identify 130 categories specific to the T genotype, just one GO term specific for the S genotype (GO:0016758 Transferase activity, belonging to *molecular function* domain) and no enriched categories for the F1. The T line showed 47, 4 and 80 GO enriched categories belonging to the *cellular component, molecular function and*

biological process main domains respectively (Supplementary Materials: Table S2). As for the common genes, enrichment analysis revealed 74 enriched GOs shared among all genotypes, 25 enriched categories in common between T and F1, 231 between T and S and just three in common between Susceptible and F1 line (Fig. 3). Among them 13, 3 and 9 categories, belonging respectively to *cellular component, molecular function, biological process*, are shared between T and F1; 88, 24 and 118 are shared between T and S genotypes; 0, 2 and 1 between S and F1 lines and 62, 3 and 8 are shared among the three genotypes (Supplementary Materials: Table S2). A deeper analysis of the enriched GO categories was able to reveal interesting biological occurrences especially in the T genotype. In particular, T specific GO enriched categories, are predominantly related to the signaling compartment (signal transmission and transduction, intracellular signaling), to the response to different stimulus (biotic stimulus, chemical stimulus, external stimulus and to reactive oxygen species) and to the fatty acids metabolic process (Fig. 4). Analyzing the signaling compartment we observed that all the GO terms belonging to such enriched categories shared mainly the same genes, all related to phosphatases, transferases, protein kinases (Fig. 5). This pronounced enhancement of the cellular signaling was not evidenced in the other two genotypes. Since the S genotype showed just an enriched GO term, we focused our attention mainly on the common enriched GO terms between T and F1 genotypes (Fig. 6). In this case, different over-represented GO terms related to carbohydrate metabolism were evidenced.

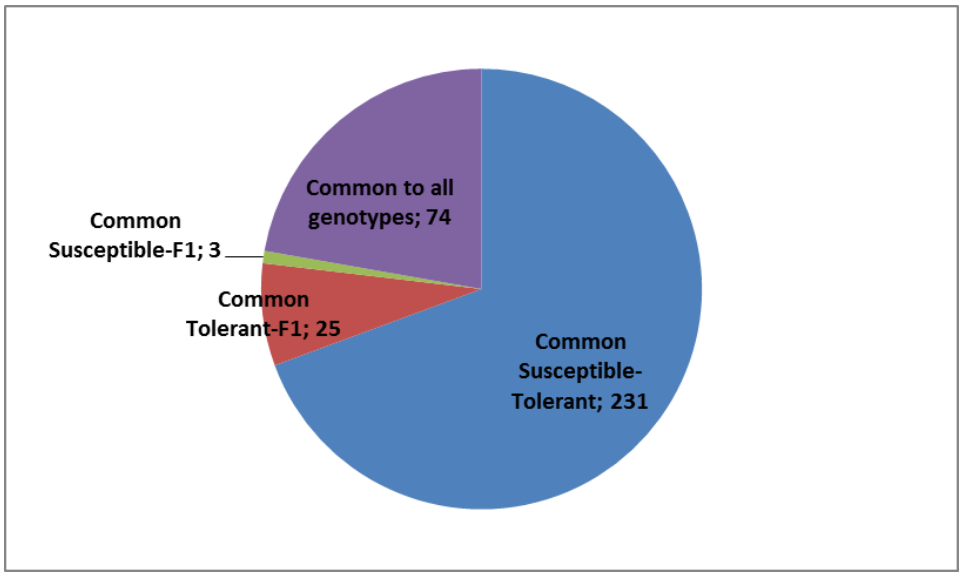

Figure 3. Apple pie showing the number of GO enriched categories in common genes among genotypes.

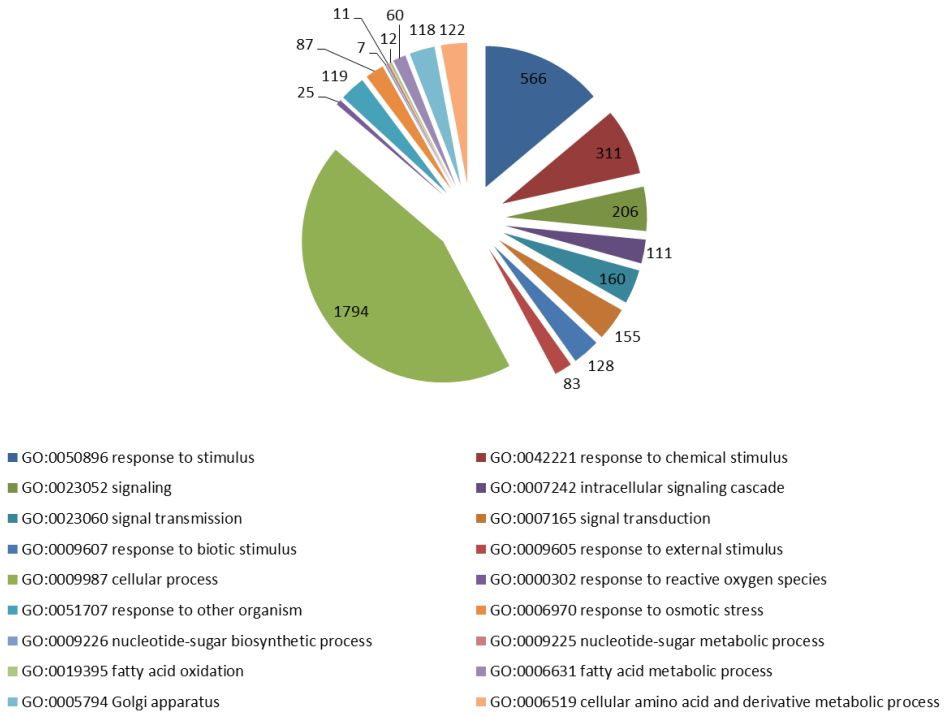

Figure 4. Apple pie showing Tolerant genotype specific GO categories.The code of each category and the corresponding associated color is reported below. Numbers of genes belonging to each GO category is indicated close to each slide.

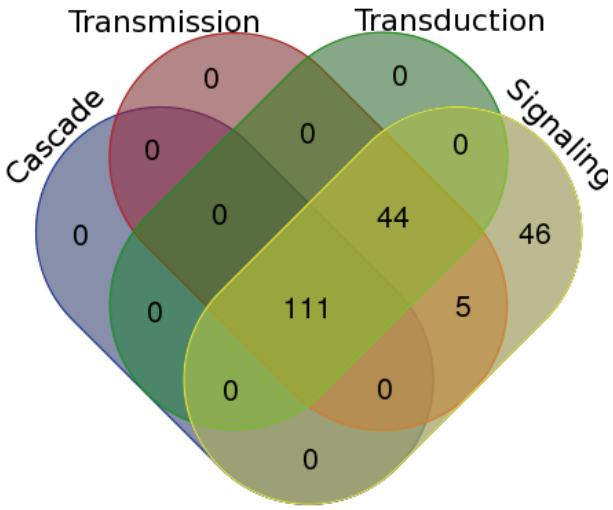

Figure 5. Venn Diagram showing the Tolerant genotype genes (indicated as numbers) in common among several GO enriched categories belonging the 'signaling' compartment.

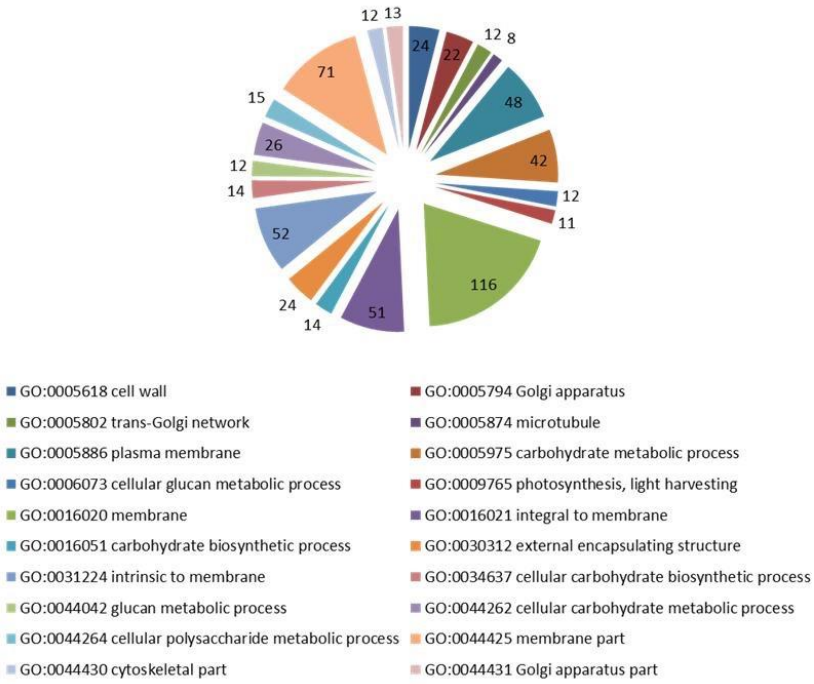

Figure 6. Apple pie showing GO enriched categories for T/F1 common genes. The code of each category and the corresponding associated color is reported below. Numbers of genes belonging to each GO category is indicated close to each slide.

3.1.3.2 Pathways reconstruction

Differentially expressed genes were mapped onto pathways to obtain an overview of tomato genes involved in the *T. absoluta* response. The *MapMan* 3.0.0 tool allowed to assign the DEGs to 35 functional classes, referred as BINs (Thimm et al., 2004; Usadel et al., 2009), with more emphasis on classes directly involved in the defense mechanisms and in the response to the herbivore feeding. Particularly, categories like 'RNA', 'PROTEINS' or 'SIGNALING' displayed the highest number of genes in all the analyzed genotypes (Fig. 7). However, a conspicuous number of DEGs was assigned to the 'Unknown/Not assigned' category (T, 2074; S, 1006; F1, 298), *Plant MetGenMap*, allowed us to match the data of gene expression changes with challenged pathways and to visualize the modification induced in a biochemical pathway contest (Joung et al., 2009). Crossing data such information an attempt was made to identify key metabolic reactions, often involved in basic cellular functions, modulated during the herbivore attack. Large enzyme families [cytochtrome p450; glycoside hydrolases; proteases], primary and secondary metabolism, transcription factors and stress-related categories [signaling; oxidative burst; hormone metabolism] were challenged and described as follow.

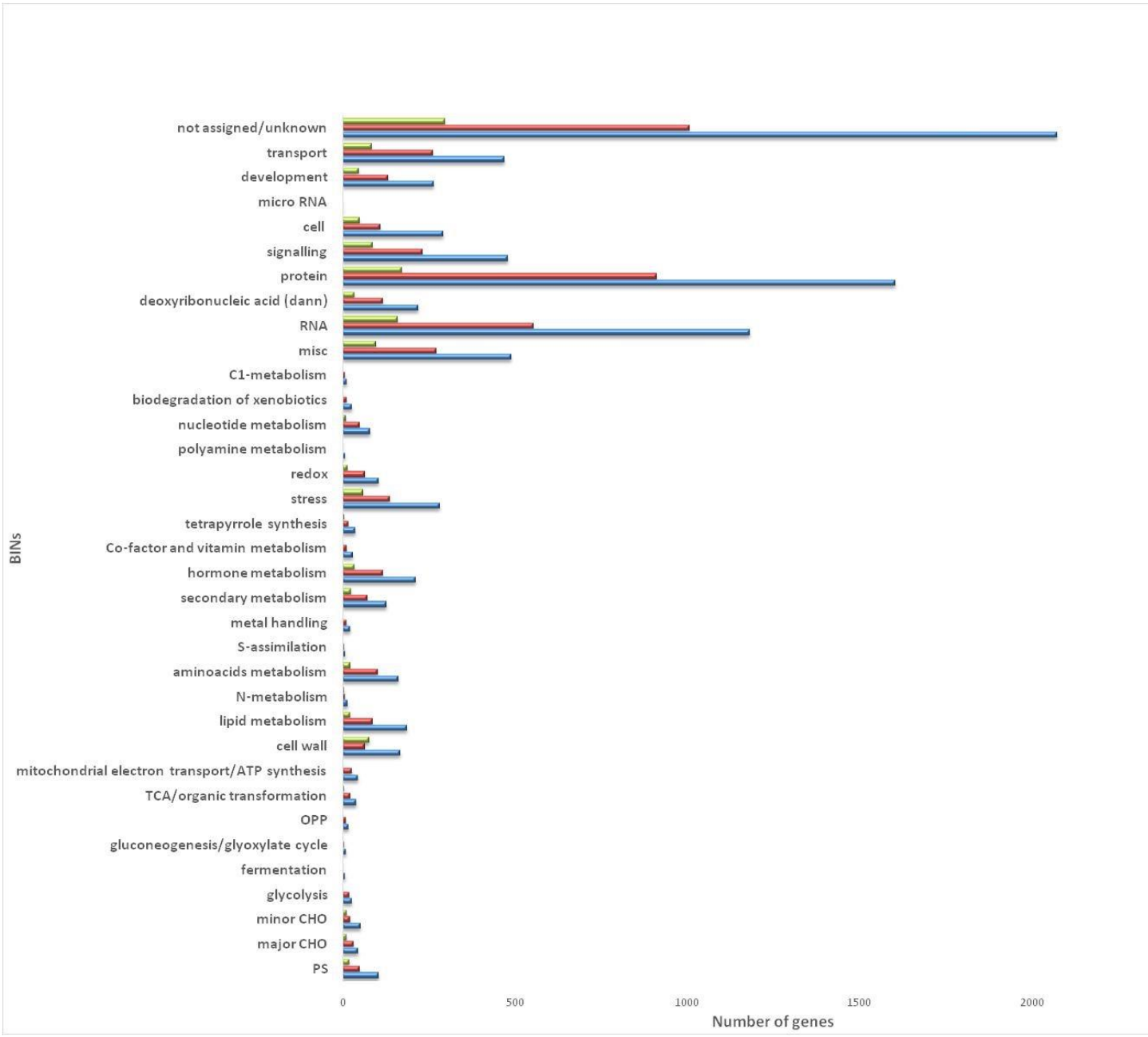

Figure 7. MapMan 3.0 DEGs analysis. Mapped BIN categories for T, S and F1 genotypes are showed on the y-axis; number of mapped DEGs on the x-axis.

LARGE ENZYME FAMILIES

Among the categories mapped with MapMan tool, we focused our attention on those related to the cellular functioning, underlying that large enzyme families could be putatively involved in the tomato-*T. absoluta* interaction. Among them proteases, glycoside hydrolases and enzymes involved in the cytochrome p450 activation were more deeply investigated. The cytochrome p450 complex is

known to be involved in some reactions connected with plant resistance and/or responses to biotic and abiotic stress (Schuler, 2010; Irmisch et al., 2013, Manzo et al., 2016). In T genotype, genes coding for enzymes of the cythocrome p450 complex resulted to be up-regulated mainly in the phenylpropanoids pathway (CYP84A1; CYP98A3), flavonoid biosynthesis (CYP75B1), terpenoids pathway (CYP88A4), abscisic acid production (CYP707A4) and in jasmonic acid pathway (CYP86A8). The S genotype shared just one up-regulated cythocrome p450 gene with T (CYP75B1 in flavonoid biosynthesis, Solyc03g122350.2), while all the others common genes resulted down-regulated. In F1 were found few up-regulated cytochrome p450 genes, mostly related to oxide-reduction reactions of photosynthesis. A strong up-regulation of genes related to protease inhibitors (PIs) was revealed by MapMan analysis in the 'protein' compartment of all the three genotypes and furthermore analyzed. As expected the tolerant line showed the highest number of up-regulated PIs; particularly serine and cysteine-type peptidases, subtilases, metallo-proteases and aspartyl carboxy-peptidases. Another category identified through the DEGs pathway mapping was related to the cell wall metabolism, particularly Glycoside Hydrolases (GH) resulted to be highly expressed and up-regulated in all genotypes.

PRIMARY AND SECONDARY METABOLISM RECONFIGURATION

A plant's resistance response to insect feeding is usually coordinated by the integration of different signals induced by wounding and insect-specific elicitors, resulting in a complex rearrangement of primary and secondary metabolism. Hence, we deeply analyzed the major switching in primary and secondary metabolism that could lead to the understanding of tomato responses to *T. absoluta*. Among the primary metabolism, a high number of DEGs mapped to BINs referred particularly to photosynthesis (PS) reactions, lipid metabolism and minor and major carbohydrates (CHO) metabolism. Our analysis of PS-related BINs revealed no particular down-regulation in PS-gene expression among the three genotypes, especially in T. By contrast fatty acids (FA) synthesis and elongation, phospholipid synthesis, reactions of beta-oxidation and FA desaturation were highly challenged. These processes are well known to be involved in plant-insect interactions, particularly in the biosynthesis of Jasmonic Acid (JA), the production of green leaf volatile compounds (GLVs) and the biosynthesis of Acylsugars (AS). The T genotype showed an abundance of DE genes related to JA biosynthetic pathway. JA is a signaling molecule, whose activity in plant-insect interactions has been well elucidated (Erb et al., 2012; Grinberg Yaari et al., 2015). The JA pathway seems to play a pivotal role in the response to *T. absoluta*, since several DE genes involved in the octadecanoid pathway for JA biosynthesis were found in our genotypes. In the Tolerant line different Fatty Acid Desaturases (FAD) involved in the production of the major precursor of JA,

alpha-linolenic acid (Schaller et al., 2005) were up-regulated (FAD2, FAD3 and FAD6). Chloroplastic lipoxygenases (LOX), catalyzing the initial steps of JA synthesis by adding molecular oxygen to linolenic acid (18:3), resulted also up-regulated. Subsequent reactions of conversion into 12-oxo-phytodienoic acid (12-oxo-PDA), catalyzed by Allene oxide synthase (AOS, Solyc11g069800) and Allene oxide cyclase (AOC, Solyc02g085730), together with the last chloroplastic step (12-oxophytodienoic Acid Reductase, OPR, Solyc07g007870), resulted widely up-regulated too. Peroxisomal beta-oxidation catalyzing the last step in JA biosynthesis resulted up-regulated too by the presence of a multifunctional protein (MFP) involved in the reaction. Furthermore, the synthesis of Methyl-Jasmonate (Me-JA) (JA-o-methyltransferase, Solyc04g080660.2) resulted highly up-regulated. MeJA is the volatile counterpart of JA (Fig. 8, panel a) and could be easily diffused in plant organs. Differences in JA biosynthetic pathway were evidenced in the other two genotypes. Particularly, the lack of up-regulated genes in S genotype (Fig. 8, panel b) during the last steps of JA biosynthesis and the presence of a down-regulated 12-oxophytodienoic Acid Reductase (Solyc10g086220.1) and a down-regulated Acyl-CoA oxidase 2 (ACX, Solyc04g054890.2), involved in the beta-oxidation of JA, could explain the main differences with the T genotype. Furthermore, FAD genes resulted present both in T and S, but the FC expression level in the T genotype is higher than the in S one. F1 hybrid showed just few up-regulated genes involved (FAD2 and ACX) and, most of them, were in common with the Tolerant genotype. Different DE genes were also mapped to biosynthetic pathways that could be connected to the production and formation of Acylsugars (aliphatic esters of sucrose and glucose), allochemicals that can confer resistance to a large number of arthropod pests, including the tomato pinworm, *T. absoluta*. Biochemical pathways for the formation of these compounds involves reactions of esterification of a sugar-based moiety with a fatty acid side chain. The branched-chain fatty acids utilized in acylsugars biosynthesis are derived from leucine, isoleucine, and valine (van der Hoeven & Steffens, 2000) and subsequently attached to UDP-glucose by a glycosyltransferase forming 1-O-acylglucose (Ghangas & Steffens, 1993). Further additions to the structure are catalyzed by acyltransferases, a group of enzymes whose involvement has been also characterized in tomato (Schilmiller et al, 2012). Among our genotypes, different genes putatively related to acylsugars pathways (glycolsytransferases, acyltransferases and UDP-glucuronosyltransferases) have been identified and resulted to be differentially expressed. Furthermore, super-pathways of leucine, isoleucine and valine resulted to be extremely up-regulated in the T genotype and less in the other two. Apparently, the alpha-ketoacid elongation (α-KAE) pathways seem to be involved in production of these allochemicals too. Our DEGs mapping is consistent with this finding since

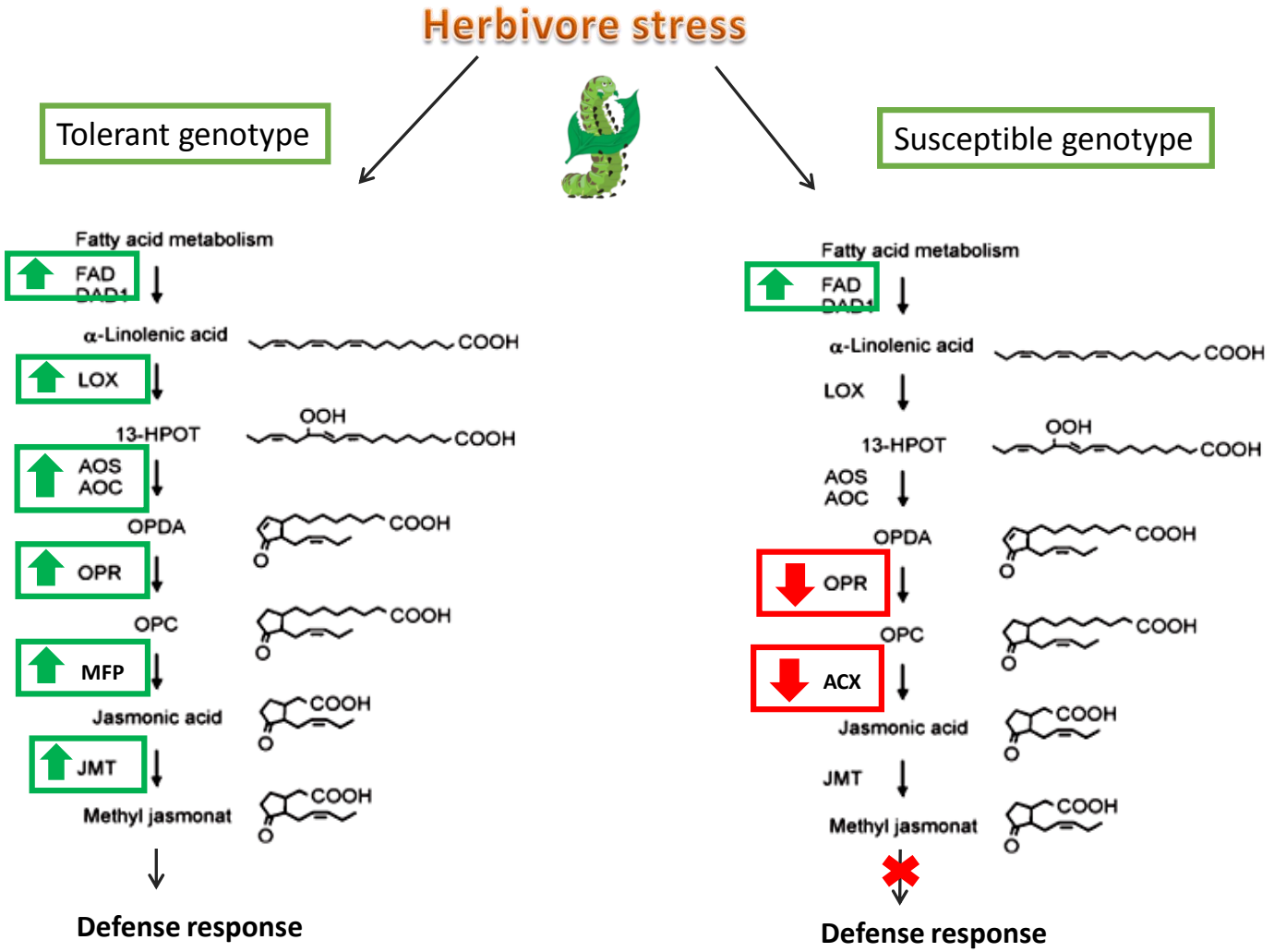

Figure 8. Transcripts activated (green arrows) or inhibited (red arrows) in the JA pathway in the tolerant (left, a) and susceptible (right, b) genotypes. In the latter the red cross indicates the inability of the susceptible line to activate a defense response.

different 3-ketoacyl-CoA synthases (KCS) involved in these kind of reactions were find up-regulated in the T genotype (KCS11, KCS3, KCS4).

Interestingly, a number of genes mapped to minor and major CHO metabolism belongs to some of the above mentioned glycosyltransferases involved in the acyl sugars formation. The re-arrangement of the secondary metabolism in response to the herbivore feeding has been assessed with DE genes mapped to isoprenoids, phenylpropanoids and flavonoids biosynthetic pathways. Among isoprenoids, terpenes are probably the largest and structurally most diverse class of plant metabolites, with a primary role in plant defense (Tholl, 2006). In particular, volatile terpenoids are the most abundant metabolites in tomato vegetative tissues and particularly in trichomes, playing a major role in resistance against herbivores. DEGs mapping revealed different genes involved in volatile terpenoids synthesis. In particular, two (E)-Beta-ocimene synthases (Solyc01g105890.2 and Solyc01g105920.2) were strongly up-regulated in the T genotype. Other up-regulated terpene synthases, mapping in the pathway of ent-kaurene biosynthesis, a precursor of gibberellin, in the tolerant genotype were detected. Furthermore the T line showed DEGs involved in the biosynthesis of other kinds of terpenes, particularly diterpenoids (via MEP/mevalonate pathways), hemiterpenes and triterpenoids (saponin biosynthesis). As for the susceptible genotype, the terpenoid pathways is less represented, since just the Beta-ocimene synthase (Solyc01g105920.2) was up regulated, with a very low fold change compared to T and biosynthetic pathways for di- and triterpenoids were mapped only with down-regulated genes. Analyzing the DEGs mapping of the F1 hybrid, only the monoterpenoid pathway resulted up-regulated and, also in this case, the same T's (E)-Beta-ocimene synthases (Solyc01g105890.2 and Solyc01g105920.2) were detected. Phenylpropanoids, produced by plant trichomes, have also a leading role in herbivores resistance (Glas et al., 2012). Plant shikimate pathway is the entry to the biosynthesis of phenylpropanoids and a general precursor for a wide range of products, including anthocyanins, flavonoids, lignin and phenylpropenes (Glas et al., 2012). Transcripts mapping revealed an extremely high up-regulation of genes encoding for enzymes in the phenylpropanoids core pathway. Particularly for the T genotype, up-regulated PAL2 (phenylalanine ammonia lyase, Solyc10g086180.1) gene and an up-regulated 4CL 1 (4-coumarate CoA ligase, Solyc08g076300.2) were detected. Furthermore, up-regulated transcripts related to enzymes of the cytochrome p450 complex were found, mainly involved in the branch of chlorogenic acid production, a compound whose involvement in plant-herbivores interactions is well elucidated (Leiss et al., 2009 a). Moreover, a wide range of other reactions connected to the core of phenylpropanoids pathway were activated in the Tolerant genotype. L-phenylalanine produced after the shikimic acid, can be the substrate of decarboxylases and alcohol dehydrogenases to obtain, respectively, volatile phenylacetaldeide and phenylethanol, two volatile

compounds generally involved in the attraction of herbivores parasitoids (Bengtsson et al., 2006). Up-regulated transcripts for these enzymes were found in the T genotype (Decarboxylase family proteins: Solyc08g006740, Solyc08g006750, Solyc08g068600, Solyc08g068610, Solyc08g068670, Solyc08g068680; Alcohol dehydrogenases: Solyc09g091700, Solyc08g083280). Biosynthesis of benzenoids and of methyl-eugenol were up-regulated too, since two benzoyl transferases (Solyc07g049660; Solyc05g015800) and a eugenol-O-methyltransferase (EOM, Solyc10g008120.2) resulted strongly up-regulated in T genotype.

Susceptible genotype seems less capable to deal with this reaction framework during the chewing pest feeding, since it showed several down-regulated genes in the phenylpropanoid pathway core reactions. In the shikimate branch, PAL 2 gene (Solyc03g042560.1) resulted up-regulated but, differently from T genotype, genes coding for the subsequent reactions resulted down-regulated (4CL, Solyc11g069050.1; Solyc03g097030.2). Noteworthy we were not able to find DEGs involved in benzenoids and methyl-eugenol production. Such occurrence suggests that such pathways are key to the tolerance to *T. absoluta*. Similarly to the T genotype, the S seems to activate genes related to the chlorogenic acid biosynthesis (up-regulated cythocrome p450 transcripts), as well as for the production of phenylethanol and phenylacetaldeide. The F1 hybrid genotype seems to activate the same step of phenylpropanoid pathway evidenced in the Tolerant parental line suggesting that it inherited all the key genes of the tolerant line elucidated before.

TRANSCRIPTION FACTORS

Thanks to MapMan categorization we were able to identify and map hundreds of TFs activated in the three genotypes during *T. absoluta* disturbance. For instance, different genes coding for AP2/EREBP (APETALA2/Ethylene responsive binding), ARF (Auxin responsive), AUX/IAA family, bHLH, bZIP, Zinc finger, MYB-domain and MYB-related TFs, NAC domain and WRKYs were found. The tolerant genotype showed more than 1000 TFs challanged, most of which up-regulated. A high number (384) of those TFs was shared with S genotype. Among these a bHLH transcription factor (*SlbHLH150*, Solyc09g065100.1) was up-regulated in T and down-regulated in S. It is part of the 159 member basic helix-loop-helix gene family, that is known to play important role in physiological, developmental and metabolic processes in tomato (Sun et al., 2015). Furthermore, in the T genotype TFs belonging to ARF and AUX/IAA families, that are strongly correlated with the growth and differentiation of tomato trichomes,were up-regulated (Deng et al., 2012). Such finding could explain the difference in response against the herbivore attack in the two genotypes. Few TFs were activate in F1 genotype and most of them were shared with the T genotype. Among them, AP2/ERF, MYB family and Auxin related TFs.

STRESS-RELATED CATEGORIES

In our study a conspicuous number of DEGs potentially involved in *T. absoluta* stress response were mapped in compartments related to receptors and signaling (T, 480; S, 231; F1, 88), hormone metabolism (T, 211; S, 117; F1, 34), disease resistance proteins (T, 87; S, 50; F1, 23) and oxidative burst (T, 104; S, 65; F1, 14). Perception of damage and subsequent signaling cascade resulted to be particularly enhanced since a whole plethora of genes were found among receptor like kinases (RLK), leucine-rich repeat (LRR), plasmodesmata located proteins (PDLP 7), flagellin sensitive protein (FLS2). In particular, a serine/threonine-protein kinase receptor (Solyc05g006570.1) involved in the perception of systemin was up-regulated only in tolerant genotype. Indeed, tolerant genotype showed the highest number of receptor kinases (166) and most of them were up-regulated, differently in the S genotype (73 in total) most of them are down-regulated. F1 shares just 15 up-regulated genes with the T genotype and all of them with a higher FC value. Signaling cascade activated after the perception was evidenced by different GO enriched terms related to signal transmission and transduction. DEGs related to calmodulins were mapped in all the three genotypes. An enhancement of hormone metabolism particularly in the T genotype was evidenced, since 211 DEGs belonging to hormone metabolism were mapped by MapMan analysis. Besides the Jasmonic Acid, also auxins could play a major role in tomato-*T. absoluta* interaction, since different DEGs were found up-regulated in this pathway (i.e. Aldehyde oxidase, Solyc11g071620; Tryptophan synthase, Solyc01g098550; IAA-amino acid hydrolases, Solyc03g121270, Solyc06g073060and Solyc10g079640) and in related ARF transcription factors routes (see above 'Transcription Factors'). Auxins are well known to be involved in plant growth and development (Mano &Nemoto, 2012) and also in tomato trichomes differentiation and formation (Zhang et al., 2015). F1 hybrid shares a number of up-regulated genes with the T line, whilst few up-regulated auxin-related genes were found in S genotype. Thirty-three DEGs were mapped in Methyl-salicylate pathway, a volatile compound released by herbivore attacked plants and, among them, SAMT, the key gene of the pathway (S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase 1, Solyc01g081340.2), resulted strongly up-regulated only in the T genotype. The T genotype showed also different up-regulated pathogenesis related proteins (chitinases, PR-3, PR-4), Cc-Nbs-Lrr proteins, Tir-Nbs-Lrr domain, Mlo4 and ATP transmembrane receptors). The great majority of these genes resulted to be up-regulated also in the F1 hybrid; in contrast, the S genotype showed the transcription of few disease resistance proteins, most of them down-regulated. Oxidative burst related genes were also analyzed since different studies elucidate a connection between this reaction and wounding damage (Orozco-Cardenas & Ryan, 1999; Schilmiller & Howe, 2005). This mechanism seems to be active both T and S genotypes, since an up-regulation of

NADPH oxidase (*SlRbohH*, Solyc11g072800.1) and of genes involved in ROS scavenging mechanisms, such as thioredoxins and superoxide dismutases, was evidenced in both genotypes.

3.2 METABOLOMICS

3.2.1 Metabolite profiling

^{1}H NMR analysis performed on the aqueous and organic leaf extracts showed detailed metabolite profiles of Tolerant, Susceptible and F1 hybrid tomato genotypes infested with *T. absoluta*. While the organic extracts contained mainly fatty acids as the major compounds, the aqueous extracts were shown to contain metabolites belonging to different classes. Figure 9 reports the ^{1}H NMR spectra of polar fractions of the analyzed genotypes with the indication of peaks related to the major metabolites identified in the spectra. Full ^{1}H NMR assignments of the identified compounds are reported in Table 1. In particular, the presence of sucrose (SU) was observed by the appearance in the spectra of the characteristic anomeric signals at δ5.25-5.29. In addition, signals for ⟨- and®-glucose (aGLC and bGLC) and for the related glucuronic acids (aGlcU and bGlcU) were also observed. The presence of appreciable amounts of malic (MA) and shikimic (SHA) acids were also observed in the spectra with their characteristic signals (Table 1). Further signals in the spectra were those related to fatty acids both in a free form (FA) or attached to sugar residues in the acyl sugars (AS). In the left part of the spectra, signals for the aromatics cholorogenic acid (CGA) and its derivatives, neo- cholorogenic acid (nCGA) and quinic acid (FQA), were observed. Signals characteristic of the aromatic aminoacid phenylalanine (Phe), tryptophan (Trp) and tyrosine (Tyr) were also detected in this region of the spectra. Finally, signals characteristic of trigonelline (TG) and © aminobutyric acid (GABA) were detected in the spectra.

3.2.2 PCA analysis

The spectra obtained from the NMR analysis were integrated by the use of iNMR software and subjected to a detailed Principal Component Analysis (PCA) (Fig 10 a-b), in order to assess metabolomic differences among samples related to plant genotype and/or exposure to *T. absoluta*. The bi-dimensional plot of sample scores (Fig 10a) clearly separated plant genotypes. Tolerant, F1 hybrid and Susceptible lines being selectively distributed in the bottom-left, top, and bottom-right quadrants, respectively. However, a higher dispersion was observed for F1 samples, indicating a higher heterogeneity of their spectra compared to Tolerant and, especially, Susceptible genotypes samples. In addition, sample replicates exposed to the leaf herbivore *T. absoluta* were rather separated from the not-infested controls, with the latter closely grouped around the PC space center. This means that, in general, the spectral contributions of selected spectral signals [i.e. δ_H 0.4-0.6, 2.3-2.4, 2.7-2.8, 4.3-4.5, 5.0-9.0], were differently distributed among and within genotypes and, moreover, consistent differences can be observed between exposed samples and controls. In other

words, existing metabolic differences among non-infested genotypes (i.e. control samples) were amplified after the exposure to the leaf herbivore. The corresponding bi-dimensional plot of signal loadings (Fig 10b) allowed us to analyze more in detail the general trend of the association between the analyzed samples and the axis of the PC space. In particular, the first PC axis was positively associated to the signals resonating at δ_H 2.7-2.8 and δ_H 7.3-7.4, diagnostic of malic acid and phenylalanine, respectively, and negatively to a rather wide spectral region including signals resonating at δ_H 0.4-0.6, 6.2-6.4, 6.7-7.1, 7.5-7.6, 8.6-8.7, and 8.9-9.0. Such signals are diagnostic of fatty acids (δ_H 0.39-0.65), chlorogenic and neochlorogenic acids (δ_H 6.19-6.27, 7.00-7.10, 7.55-7.62), 5-O-feruloyl quinic acid (δ_H 6.27-6.36, 7.00-7.10, 7.55-7.62) and trigonelline (δ_H 8.96-9.03, 8.62-8.75).The second PC axis was related to carbohydrate content,being positively associated with the signals resonating at δ_H 5.0-5.6, characteristic of sugars such as α–glucose (δ_H 5.07-5.09) and sucrose (δ_H 5.25-5.29), and negatively with the signals resonating at δ_H 4.3-4.5, characteristic of β-glucose (δ_H 4.48-4.51), and α- and β-glucuronic acid (δ_H 4.37-4.44). A more detailed characterization of metabolites identified with the ^{1}H NMR analysis has been carried out, by comparing the association between the PC axis and the spectral signal loadings (i.e. colored arrows in the graph) with the samples scores in the PC space (i.e. sample locations in the graph) (Fig 11 a-b). In this way we were able to characterize both metabolomics of the three genotypes and the chemical changes produced after the *T. absoluta* feeding (Table 2).

Table 1. Characteristics of ^{1}H chemical shift (d) and coupling constants (J) in metabolites identified using ^{1}H NMR spectra and reference compounds.

COMPOUND	δ(ppm)	multiplicity (J, Hz)
sucrose (SU)	5.25-5.29	
alpha-glucose (aGlc)	5.07-5.09	d(J,3)
beta-glucose (bGlc)	4.48-4.51	d(J,7)
alpha-glucuronic acid (aGlcU)	4.37-4.44	
beta-glucuronic acid (bGlcU)	4.37-4.44	
malic acid (MA)	2.72-2.81	
	4,4	
shikimic acid (SHA)	4.30-4.44	
	6.71-6.82	
fatty acid (FA)	0.39-0.65	
trigonelline (TG)	8.96-9.03	
	8.62-8.75	
GABA	2.36-2.42	
phenylalanine	7.34-7.41	
chlorogenic acid (CGA)	6.19-6.27	d(J,16)
	7.00-7.10	bd(J,9)
	7.41-7.55	d(J, 16)
neochlorogenic acid (nCGA)	6.19-6.27	d(J, 16)
	7.00-7.10	bd(J,9)
	7.42-7.67	
5-O-feruloyl quinic acid (FQA)	6.27-6.36	d(J,16)
	7.00-7.10	bd(J,9)
	7.55-7.62	d(J,16)

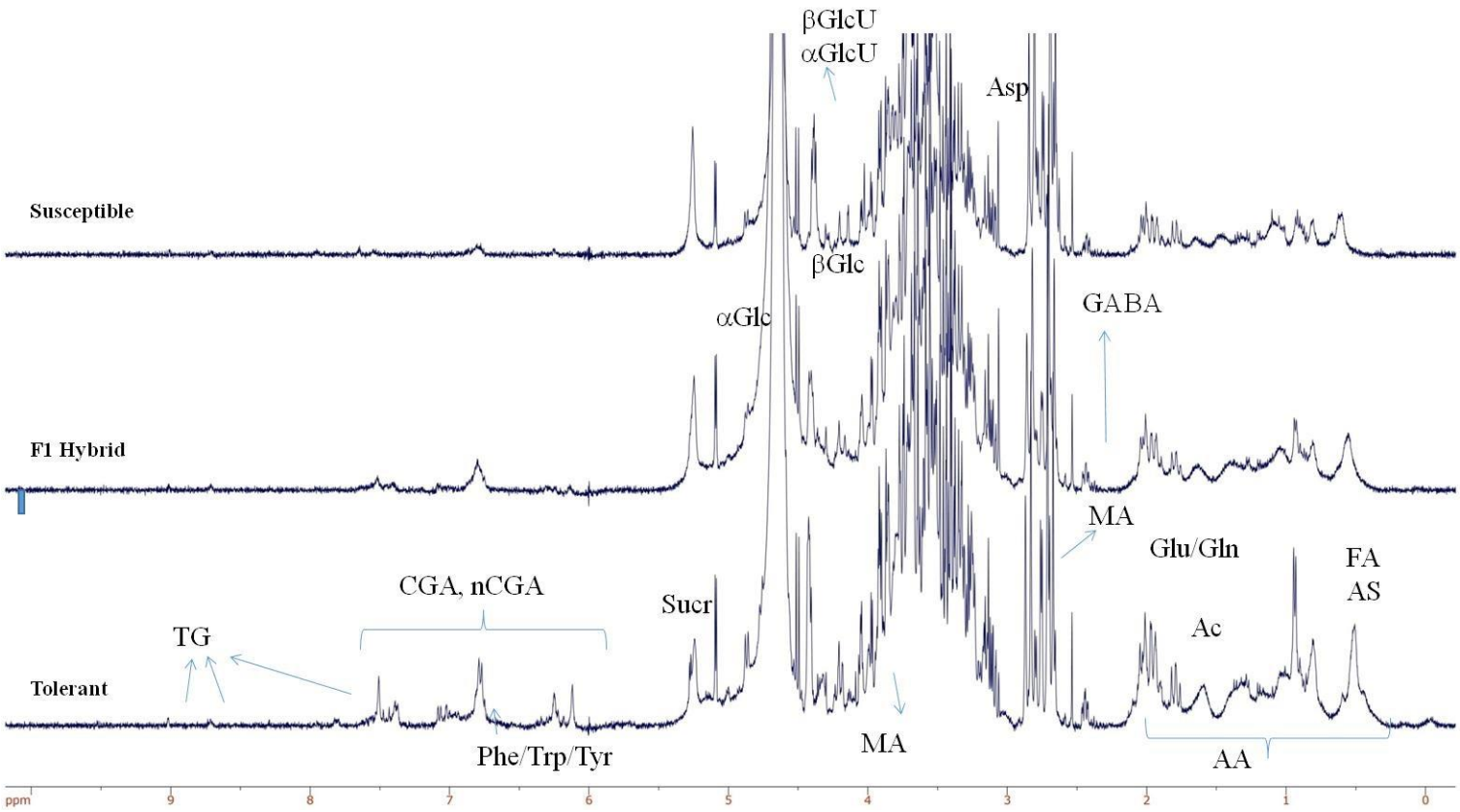

Figure 9. Full 1H NMR spectrum (D2O, 400 MHz) of Susceptible, Tolerant and F1 hybrid tomato genotypes after *Tuta absoluta* disturbance.

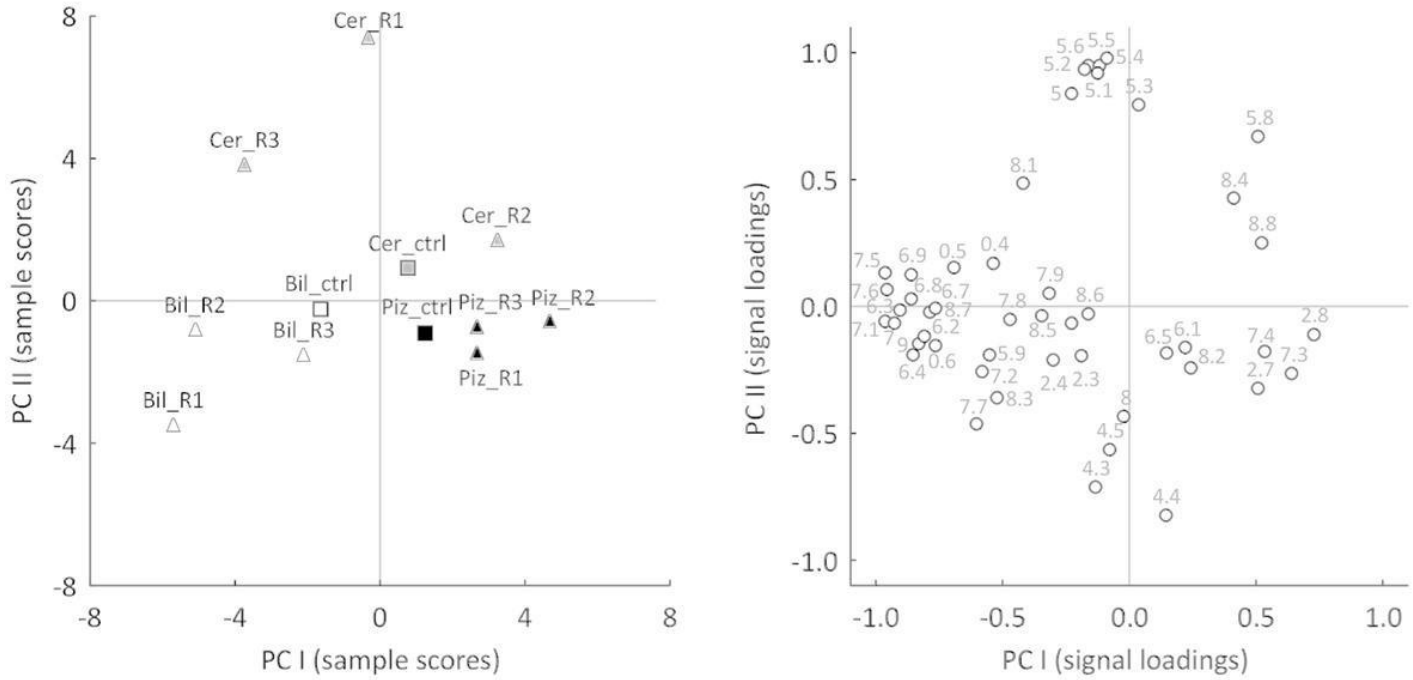

Figure 10. PCA of selected reference ^{1}H NMR spectral signals for polar fractions. Left (a): plot of sample scores. Symbol color and shape indicate genotypes (white, T; grey: F1; black: S) and treatment (triangles: infested; squares: control), respectively. Right (b): plot of signal loadings. Data labels indicate sample ID and signal resonance (ppm), respectively.

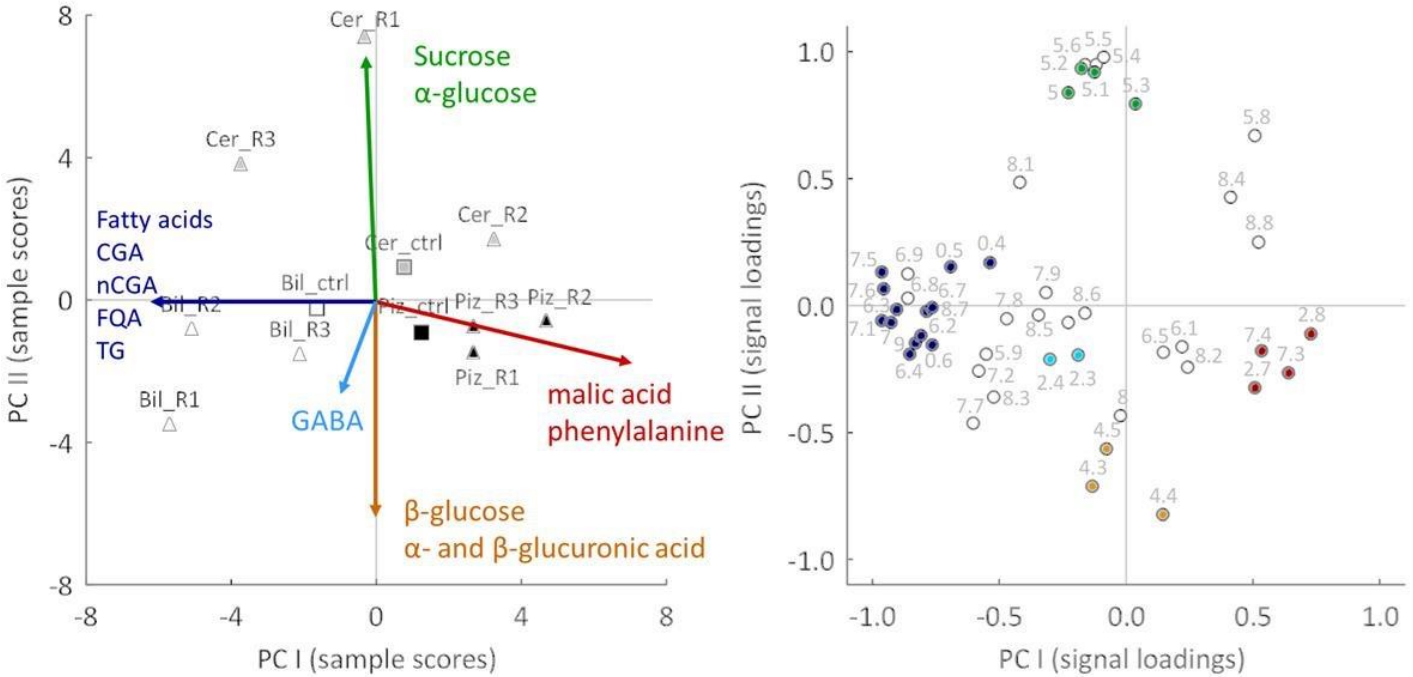

Figure 11. (panel a, b): Association between the PC axis and the spectral signal loadings (i.e. colored arrows in the graph) with the samples scores in the PC space (i.e. sample locations in the graph) in order to unveil the chemical changes in the three genotypes.

Table 2. Detailed content of metabolites evidenced in Tolerant, Susceptible and F1 hybrid genotypes in both infested and not infested samples (CGA = Chlorogenic Acid; n CGA = neo Chlorogenic acid; FQA = feruloylquinic acid; aGLc = alpha-glucose; SU = sucrose; bGLc = beta-glucose; a- /b- GlcU = alpha-/beta-glucuronic acids; GABA = gamma-aminobutyric acid).

Genotype	Condition	Phenylanine (7.3-7.4 ppm)	Malic Acid (2.7-2.8 ppm)	Fatty Acids (0.4-0.6 ppm)	CGA, n CGA, FQA (6.2-6.4; 7.0-7.1; 7.5-7.6 ppm)	Trigonelline (8.6-8.7; 8.9-9.0 ppm)	aGlc/SU (5.0-5.3 ppm)	bGlc/ a-/b-GlcU (4.3-4.5 ppm)	GABA (2.3-2.4 ppm)
Tolerant	infested	0	54.1	37.0	22.8	0.91	46.9	51.2	4.5
	not infested	0	56.9	32.9	17.8	0.04	44.1	41.9	0.1
F1	infested	0.04	55.1	20.9	10.3	0.38	96.8	36.4	3.3
	not infested	0	56.3	16.8	4.8	0	60.3	26.7	0.5
Susceptible	infested	0.17	124.9	8.6	2.3	0.24	60.5	58.3	5.2
	not infested	0.30	103.5	7.6	0.8	0	53.1	49.0	0.4

First, S samples showed a higher content of malic acid (MA) and phenylalanine (Phe), which also increased particularly after the exposure to the herbivore (Tab. 2). Also the T genotype showed MA production, but in smaller amounts compared to the S line. On the contrary, the T genotype showed an abundance in organic acids, including Fatty Acids (FA) and Acylsugars (AS), Chlorogenic acid (CGA), neo-chlorogenic acid (nCGA) and feruloylquinic acid (FQA), detected in very small amounts in the Susceptible genotype. The content of these organic compounds was very low in control samples, indicating a clear link between the exposure and the metabolic pathways related to such specific organic molecules. Higher content of the piridinic alkaloid Trigonelline (T) was detected too in all of the three genotypes, with the T showing the highest change of abundance. The role of this alkaloid could be considered for further investigation in plant-herbivores interactions, and this is probably the first time that such metabolite has been isolated in tomato after the exposure to a lepidopteran insect feeding. The F1 genotype was distinctively different from the other two genotypes since it showed higher amounts of α-glucose and sucrose and lower content in β-glucose and α- and β-glucuronic acids, whereas both T and S genotypes showed similar amounts of these carbohydrates. Furthermore, carbohydrates contents were always higher in infested samples than the non-infested, for all the three genotypes, indicating some connections between this aspect and the response to *T. absoluta*. Interestingly, signals related to GABA (γ-aminobutyric acid) (δ_H2.3-2.4) were relatively much higher in infested samples of all genotypes compared to the corresponding non-infested controls.

3.3 GENOMICS

3.3.1 Variant Calling and annotation

In order to detect structural differences in our genotypes and to assess if these differences could influence the expression of genes involved in the response to *T. absoluta*, a calling of SNPs and short Insertions and Deletions (InDels) was performed on transcripts obtained from the sequencing of RNA. Detection of these variants was carried out on infested and non-infested samples from Tolerant, Susceptible and F1 genotypes. The standard approach to perform this variant calling was to map the raw sequence reads obtained from the RNA sequencing against the *S. lycopersicum* reference genome (SL 2.5). The transcriptomic variants were then predicted and annotated using SNPeff tool. The total number of variants distributed for each of the 12 tomato chromosomes (including chromosome 0) is shown in Table 3. Infested samples for each genotype showed an higher number of variants compared to those detected in non-infested samples. This is probably due to the higher number of expressed genes in the infested samples. Chromosomes 01 and 07, in each of the analyzed samples, revealed the highest number of variants. Considering the total number of variants for each sample, the S_infested and F1_infested showed the highest number of variations. Variants were furthermore catalogued and analyzed (Tab 4). The SNPs represent the most abundant sources of variation in our tomato genotypes. S; the highest number of SNPs was detected in S infested sample (293890) and F1 infested sample (265612), while Tolerant genotype showed, respectively for infested and non-infested samples, around 205000 and 130000 SNPs. Sources of variation associated to Insertions and Deletions are less representative, since a very low number was evidenced. Even in this case, the highest number of Insertions was detected on S_infested (13732) and on F1_infested (12317). Same for Deletions, S_infested and F1_infested showed respectively 11236 and 9585 variants. A functional classification helped us to assess the impact of the detected variants, particularly if they resulted to be synonymous or non-synonimous. Among the latter, missense and nonsense variants are those whose effect results to be deleterious, leading to a change in the aminoacidic structure. Missense variants were detected in higher amounts than nonsense and/or silent variants. Infested sample of our tomato genotypes showed the highest number of those variants; respectively for T, S and F1, around 35000, 53000 and 50000 missense variants were detected (Tab 5). In contrast, a very low number of nonsense variants was elucidated, as shown in Table 5.

Table 3. Total number of variants/chromosome for each sample (T, S, F1 hybrid) in both infested and not infested conditions.

TOTAL NUMBER OF VARIANTS						
	T_infested	T_non infested	S_infested	S_non infested	F1_infested	F1_non infested
Chr00	2341	1597	2869	2011	2606	1764
Chr01	42927	26176	57717	37132	51093	34101
Chr02	19703	12473	12918	8017	19115	12127
Chr03	12883	8721	23302	14618	19285	13165
Chr04	30468	18879	37139	24072	35729	23900
Chr05	15613	10483	37305	24517	29631	21235
Chr06	6327	4600	8679	5345	7500	5126
Chr07	44751	27761	44450	29383	44006	28644
Chr08	7530	4496	7011	4252	7093	4587
Chr09	6398	4236	21090	13258	15071	10754
Chr10	8958	5674	23812	15190	17878	12618
Chr11	16314	10663	25535	16787	21569	14814
Chr12	12169	7178	17031	10781	16938	11452
TOT number	226382	142937	318858	205363	287514	194287

Table 4. Total number of variants classified according to their type.

TOTAL NUMBER OF VARIANTS BY TYPE						
	T_infested	T_non infested	S_infested	S_non infested	F1_infested	F1_non infested
SNPs	205502	130223	293890	189825	265612	180121
INSERTIONS	11978	7461	13732	8708	12317	8158
DELETIONS	8902	5253	11236	6830	9585	6008

Table 5. Total number of variants classified according to their effect (missense, nonsense and/or silent).

TOTAL NUMBER OF VARIANTS BY FUNCTIONAL CLASSIFICATION						
	T_infested	T_non infested	S_infested	S_non infested	F1_infested	F1_non infested
Missense	35104	22976	53652	33717	50261	33667
Nonsense	1344	873	1884	1128	1734	1208
Silent	22679	15119	37741	23990	35405	23925

To gain more insights about the effect of those predicted variants on tomato responses to *Tuta absoluta*, we integrated genomic and transcriptomic data. Thanks to SNPeff tool, we were able to take advantage of the list of annotated genes affected by predicted variants and, within those genes, identify those differentially expressed according to our RNA-Seq experiment. DEGs with variants

were recorded for each genotype in each condition (if infested or not-infested) and their total number is depicted in the bar chart in Figure 12. T_infested showed the highest number of DEGs with variants (6794), followed by its control sample (non-infested) with 5954 DEGs.

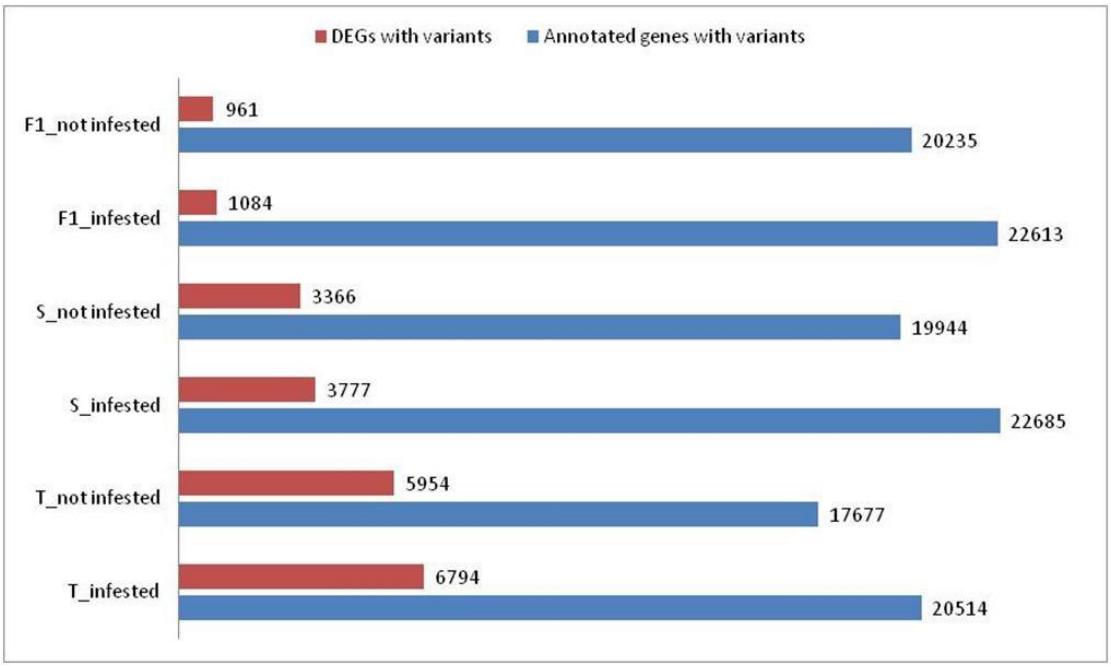

Figure 12. Total number of genes affected by variants in T, S and F1 hybrid genotypes (in both infested and not infested conditions). Blue bars represent total number of annotated genes with variants (according to SNPeff prediction); red bars indicate total number of genes differentially expressed affected by variants.

3.3.2 Identification of variants in gene classes involved in the response

To get more insights of the mean of gene variants occurring between T, S and F1 genotypes and the *S. lycopersicum* reference genome, we focused our attention on gene classes putatively involved in the response to *T. absoluta*. We choose gene classes functionally annotated with the MapMan tool, which role has been well elucidated in the trascriptomic paragraph of this manuscript (signaling compartment, transcription factors, hormone signaling, secondary metabolism, lipid and CHO metabolism, genes for oxidative burst reactions, cytochrome p450, glycoside hydrolases and proteases). All the variants were classified based on their gene location, i.e. exon and intron variants, upstream/downstream variants, splice and frameshift variants in order to obtain precisely which gene is affected by deleterious variants. The percentages of variants found in the above mentioned gene classes for each genotype are represented in the bar charts in Figure 13 (a-b-c). Among gene classes for the three genotypes a high percentage of variations is included in the exon

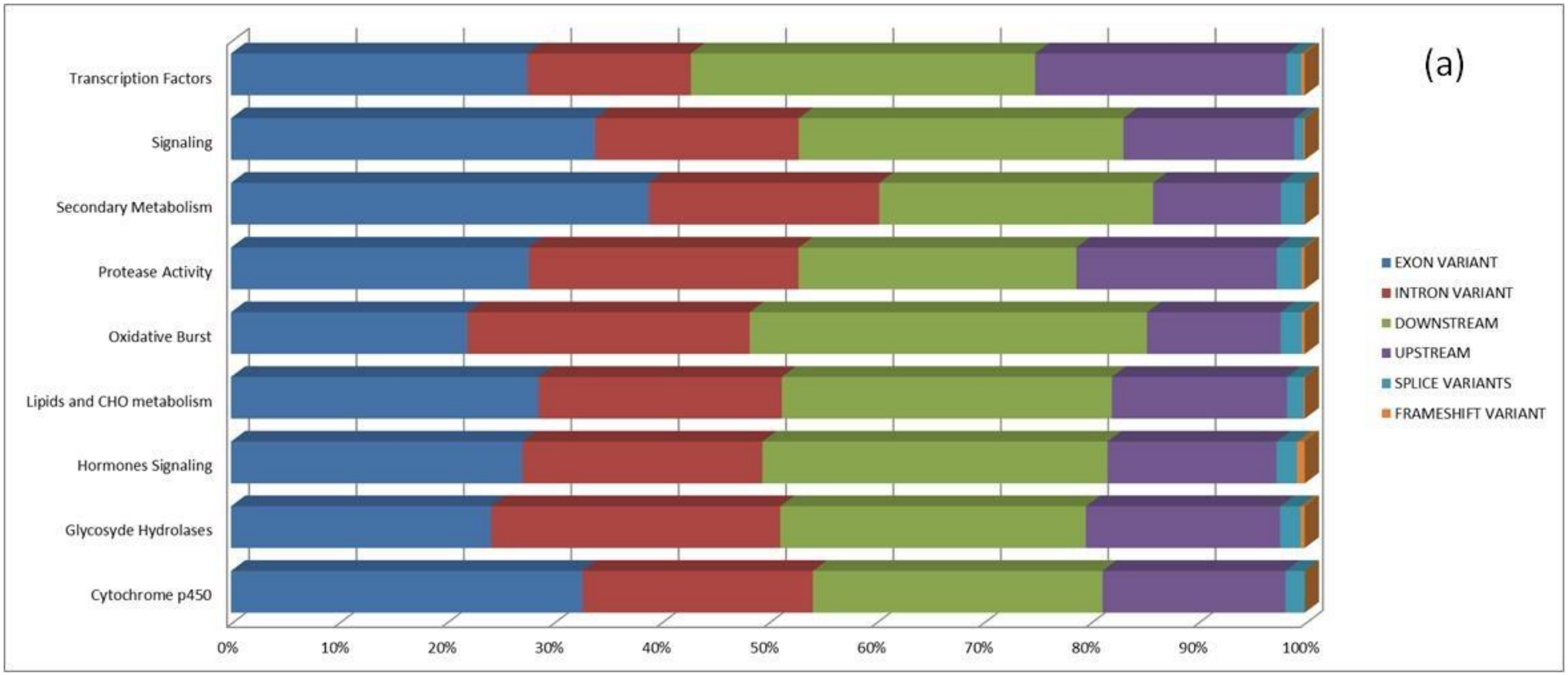

Figure 13. Panel (a), classification of variants affecting genes involved in the response to *Tuta absoluta* in the Tolerant genotype, based on their gene location.

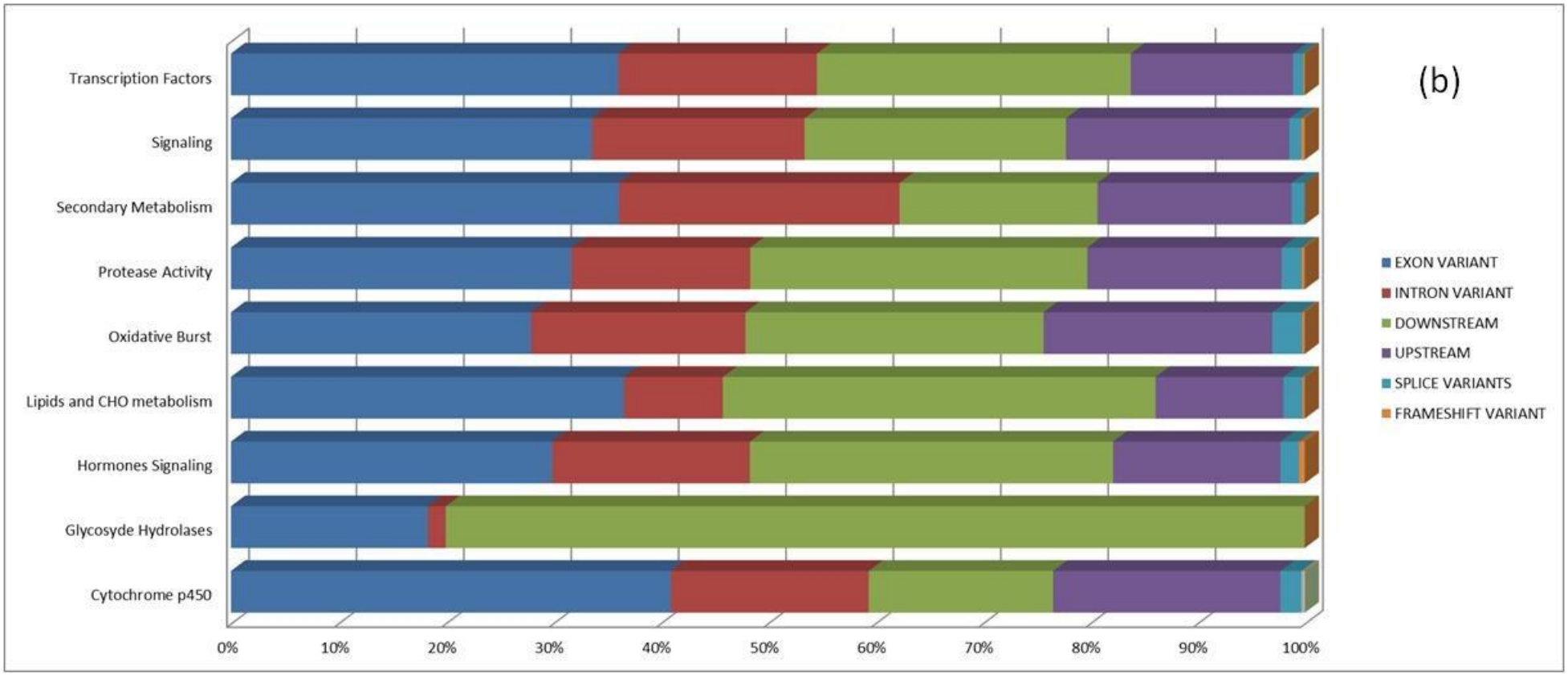

Figure 13. Panel (b), classification of variants affecting genes involved in the response to *Tuta absoluta* in the Susceptible genotype, based on their gene location.

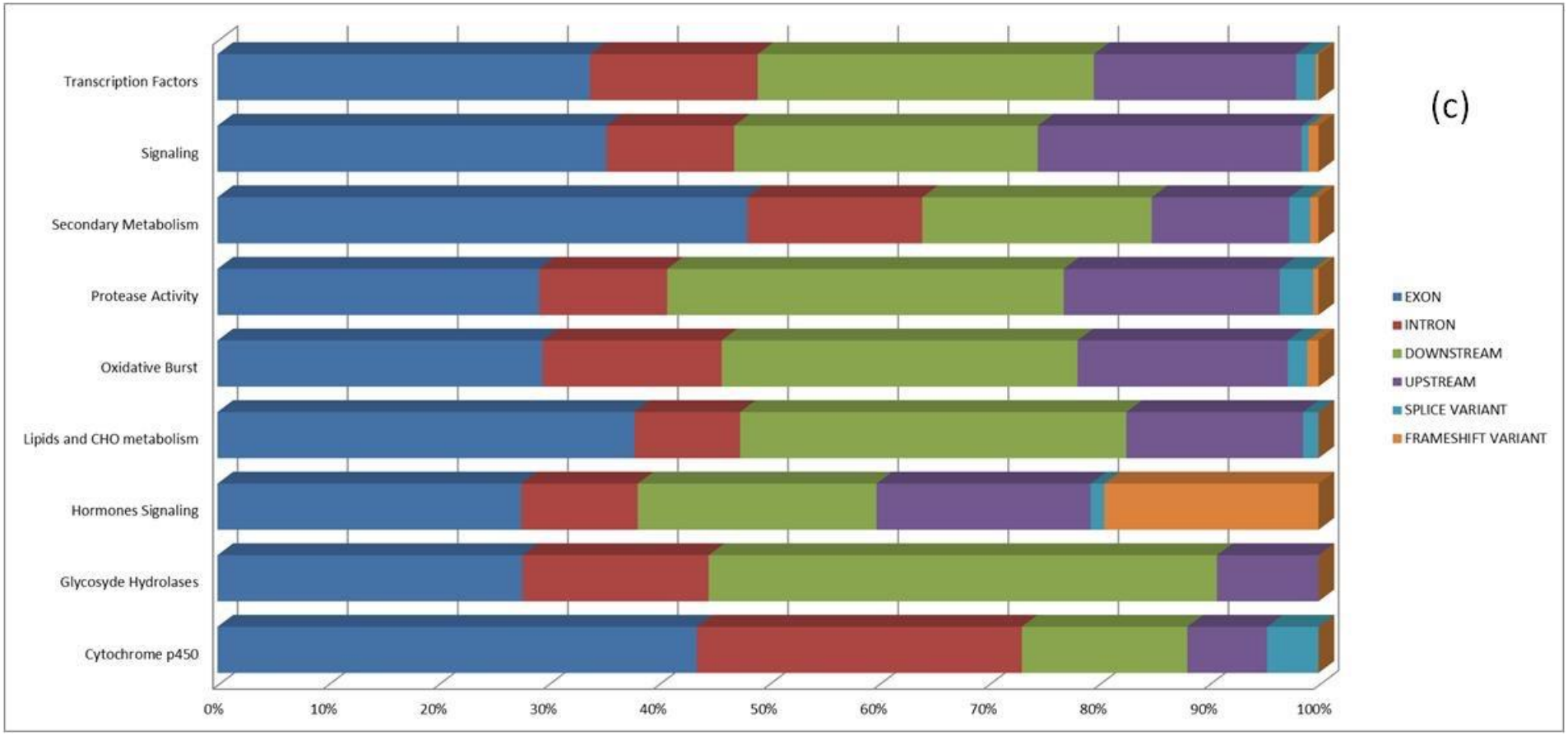

Figure 13. Panel (c), classification of variants affecting genes involved in the response to *Tuta absoluta* in the F1 hybrid genotype, based on their gene location.

region. Downstream variants are more represented in the T and S genotypes, and less in the F1; on the contrary, splicing and frameshift variants, whose effect is known to influence the final aminoacidic structure, are highly represented in F1 hormone signaling genes and poorly in the other gene classes of the two parental lines. The S genotype showed a high percentage of exon and downstream variants, particularly for the Glycoside hydrolases. Putative impact of variants has been evaluated, focusing on variations with deleterious effect, such as missense variants and/or start/stop lost/gained, frameshift and splice variants. Interestingly, we found some genes affected by those variants which are already known to be involved in the tomato-*T. absoluta* interaction (Supplementary Materials: Tabs S3-S4-S5). Deleterious variants that could explain the difference of our genotypes with the *S. lycopersicum* reference genome were detected on both T and S genotypes, especially in gene classes related to Secondary metabolism and Hormones metabolism. In T genotype genes involved in terpene biosynthesis (Solyc07g042630.2, Solyc08g005640.2, Solyc08g005680.2, Solyc08g005720.2, Solyc12g006510.1, Solyc12g006530.1) contain missense_variants; furthermore, the E-beta-ocimene synthase shared by T and S (Solyc01g105920.2) results to be affected by a stop gained variant in the susceptible line (position 93956704) leading to an aminoacidic change (p.Glu260*/c.778G>T) that could explain the difference in expression in the two genotypes. Phenylpropanoid related genes (Solyc02g093230.2, Solyc02g093250.2, Solyc04g063210.2, Solyc07g049660.2, Solyc10g008120.2) contain also missense_variants. The RNA-Seq analysis suggests that these classes of genes are strongly involved in the tomato-*T. absoluta* interaction. In particular genes such as Eugenol-o-methyltransferase (Solyc10g008120.2) and benzoyl transferase (Solyc07g049660.2), are up-regulated only in the T genotype, explaining probably the partial resistance response. Another interesting gene class affected by missense variants was the ‘cytochrome p450’. Monooxigenases belonging to this class, involved in Terpenoid and Flavonoid biosynthesis resulted to be affected by deleterious variants. On the other side, in the S genotype deleterious variants were detected on some down-regulated genes. Among these, a Lipoxygenase (Solyc01g006560.2) evidenced a variation from 1124262 to 1139475 kB and a 12-oxophytodienoate reductase (Solyc10g086220.1), evidenced a variation from 65116245 to 65126297 kB. Both those genes are involved in the JA biosynthesis and their down-regulation could be linked to the presence of missense variants that lead to some amino acidic change. F1 genotype shared some genes affected by variants with the T genotypes. Among them, an Ent-kaurene/terpenoid synthase (Solyc08g005640.2) resulted affected in the same positions (505573, 505622, 506851, and 506925) by missense_variants that lead to the same amino acidic change. Such result, let us postulate that probably this ent-kaurene synthase is one of the key gene of the interaction.

3.3.3 Variants distribution

To assess the genome distribution of variants, each chromosome has been split up in window regions of 1Mb for determining the number of variants in a given chromosome portion. The graphical representation of chromosome variants distribution (Supplementary Materials: Figure S2) allowed us to identify differences among genotypes. The 'variant-peak' regions were obtained normalizing the number of variants for region on the average number of variants in each sample. In this way we were able to identify candidate regions that showed a high number of differences and genes that could be putatively involved in the response to *T. absoluta*. In other words our goal was to test whether it is possible to reveal genes putatively involved in *T. absoluta* response, analyzing regions with a high source of variation. We identified a number of variant-peak regions across the entire T and S genotypes. Notable variant-peaks were identified in Tolerant line's chromosomes 01, 02, 08, 07 and 12. Each of these regions has been deeply analyzed, firstly for gene composition and, secondly for impact of variants affecting genes. On chromosome 01, six large peak regions (4Mb, 40-42Mb, 48Mb, 60Mb, 72Mb and 93Mb) were identified. In particular the region at 93 Mb contains two interesting genes (SlAT1 and SlAT2) involved in the acyl sugars production that will be discussed later. Genes putatively involved in the *T. absoluta* response and production of acyl sugars, were also identified in regions of chromosome 02: among them six Aldose-1-epimerase-like proteins (Solyc02g087770.2; Solyc02g087780.2; Solyc02g087790.1; Solyc02g087800.2; Solyc02g087810.2; Solyc02g087820.1), an enzyme involved in conversion of alpha-glucose to beta-glucose, and different Acyl-transferases. Analysis of variant-peaks revealed interesting results also on chromosomes 07 and 08. On the first one we identified six wound induced proteins (Solyc07g054750.1; Solyc07g054760.1; Solyc07g054770.1; Solyc07g054780.1; Solyc07g054790.1; Solyc07g054800.1), a class of genes well elucidated for their involvement in plant-herbivores responses. Furthermore, an ent-kaurene synthase (Solyc07g066670.2) and a S-adenosyl-L-methionine salicylic acid carboxyl methyltransferase-like protein (Solyc07g064990.2) were detected. Genes involved in the fatty acids biosynthesis and acyl-transferases were detected also on peak-regions in chromosomes 08 and 12. Interestingly, two Cycloartenol Synthases (Solyc12g006510.1; Solyc12g006530.1), involved in terpenoid biosynthetic processes, were identified on chr 12. T genotype showed a high percentage of genes with variants of 'Unknown function'. This particular aspect should be better analyzed in the future for providing a functional characterization of those above mentioned genes. An interesting difference with the S genotype was noticed in variants distribution. In particular, S genotype showed notable peaks on chromosomes 09, 10 and 11. Among them, only chromosome 09 revealed genes that could be putatively involved in the response to the herbivore. Moreover, differences between T and S genotypes have been

assessed looking at the variants quality composition of the identified peaks. Candidate regions detected on the Tolerant genotype didn't show particular deleterious variants, differently from the S genotype that revealed a high number of stop codon variants. With this approach we were able to identify 'variants-rich' zones on each chromosome that could explain differences between genotypes reactions to *T. absoluta* response.

3.3.4 Gene and Genome arrangements of DEGs

Cluster analysis lead us to perform an investigation of the DEGs distribution along the tomato chromosomes and to identify genes that tend to be grouped in close proximity. Gene clusters were detected on Tolerant, Susceptible and F1 hybrid genotypes. Panels a, b and c of Figure 14 illustrate the chromosome profiles of the three genotypes; down-regulated genes in *infested vs non-infested* condition are colored in pink while up-regulated DEGs are in light blue; the green colored portions represent the detected clusters. Total number of clusters, involving at least 5 genes, are reported for each chromosome on the same Figure. Both T and S genotype showed high number of clusters, particularly on chromosomes 4 and 6 while the F1 genotype showed a different pattern, since chromosomes 1 and 2 showed the highest number of clusters. . Both T and S genotypes evidenced the differential expression in the same cluster (number 1) of Chr1 of two Lipoxygenases (Solyc01g005930 and Solyc01g006560) and a FAD gene (Solyc01g006430) involved in the Jasmonic Acid biosynthesis. In T genotype other two genes involved in JA pathway, showing differential expression, are clustered together on chromosome 5. Several other classes of genes that could be involved in the partial resistance to *T. absoluta* are grouped together in the T genotype. Among these, two terpenes synthase-like (Solyc08g005640, Solyc08g005720) and a terpenoid synthase (Solyc08g005670) on cluster 1 of chr8 are grouped together with a cytochrome p450 gene (Solyc08g005650), all of them involved in the Ent-kaurene synthesis, a gibberellin precursor as well as two up-regulated genes involved in phenylacetaldeide/phenylethanol production (Solyc08g006740 and Solyc08g006750), and two UDP-glucuronosyl/UDP-glucosyltransferases (Solyc08g006330 and Solyc08g006410) putatively involved in the biosynthesis of acylsugars. Another class of genes that tends to cluster along chromosomes is the Glycoside hydrolase family, whose involvement in the herbivore response has been already elucidated.The S genotype showed an up-regulation of above mentioned genes for phenylacetaldeide/phenylethanol belonging to cluster 1 of chr8. Interestingly, a cluster of up-regulated genes related to oxidative burst reactions (Glutathione-S transferases: Solyc09g011520, Solyc09g011540, Solyc09g011550, Solyc09g011580, Solyc09g011590; Thioredoxin-like fold: Solyc09g011600, Solyc09g011630) was

found in S, on cluster 3/chr9. Three Glutathione-S transferases (Solyc09g011540, Solyc09g011580, and Solyc09g011590) were up-regulated and grouped also in T genotype, but in a different cluster.

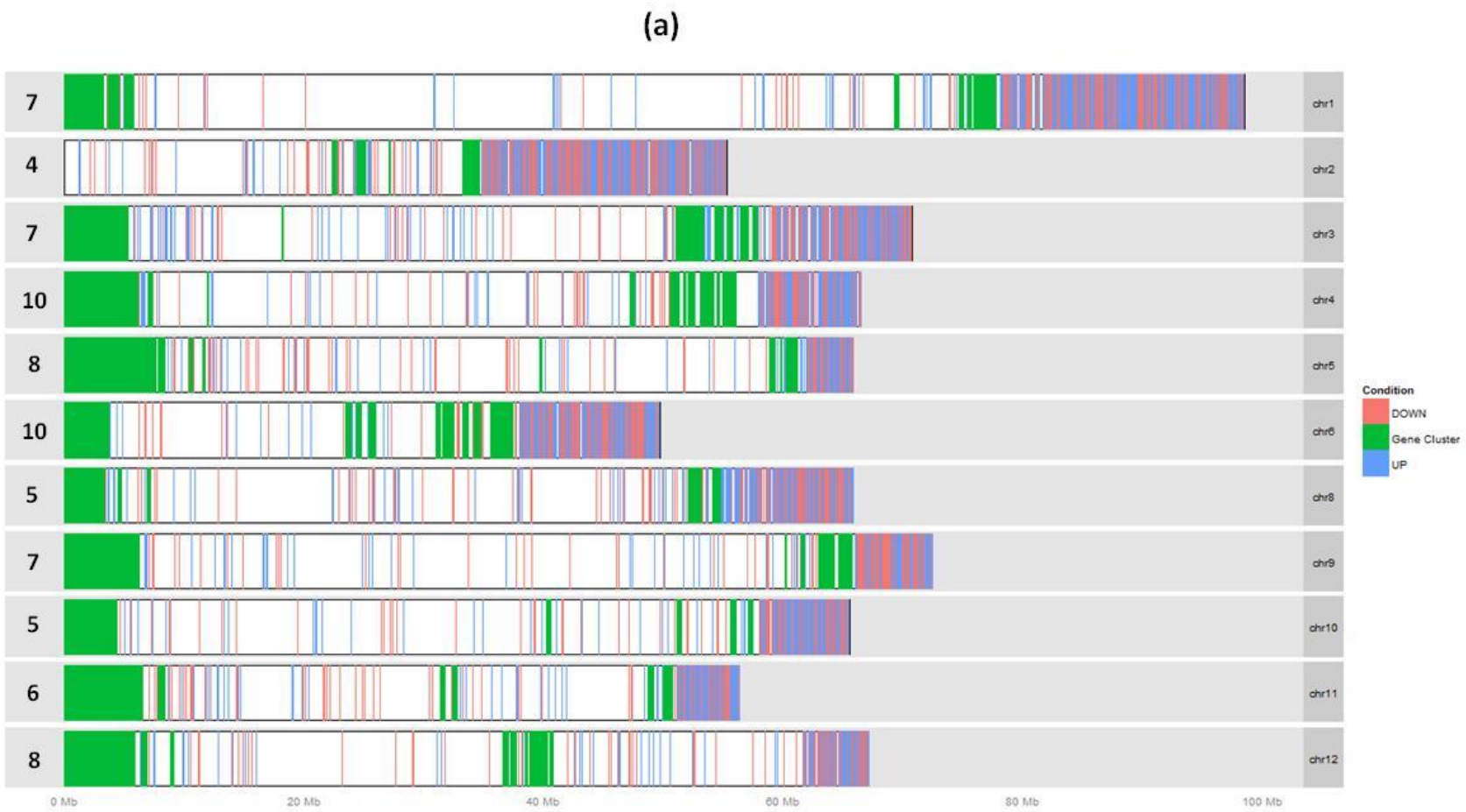

Figure 14. Panel (a), Distribution of DEGs along Tolerant chromosomes. Down-regulated genes in *infested vs non-infested* condition are colored in pink, while up-regulated DEGs are in light blue; the green colored portions represent gene clusters. Total number of identified gene clusters is presented on the left side of the chromosome.

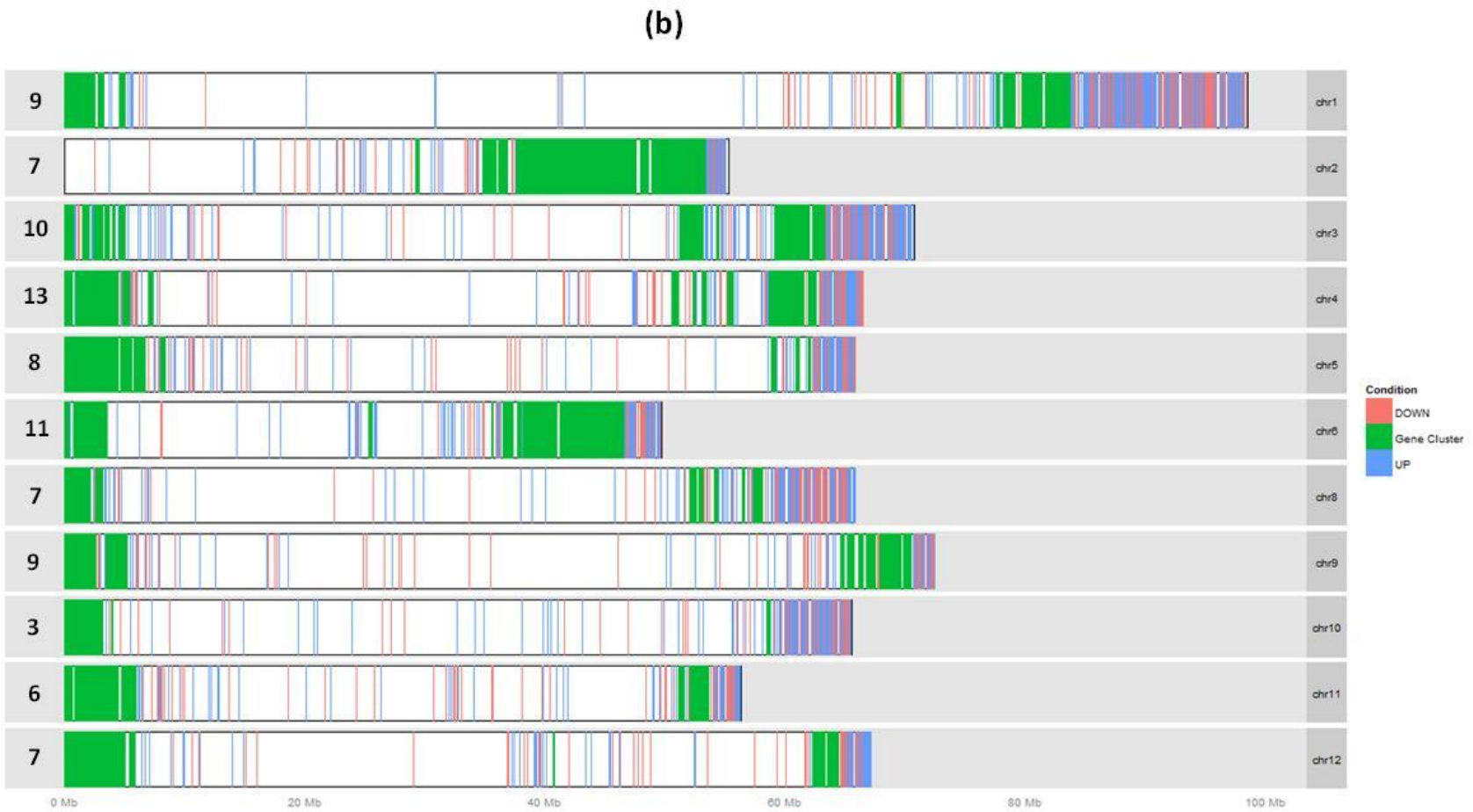

Figure 14. Panel (b), Distribution of DEGs along Susceptible chromosomes,. Down-regulated genes in *infested vs non-infested* condition are colored in pink, while up-regulated DEGs are in light blue; the green colored portions represent gene clusters. Total number of identified gene clusters is presented on the left side of the chromosome.

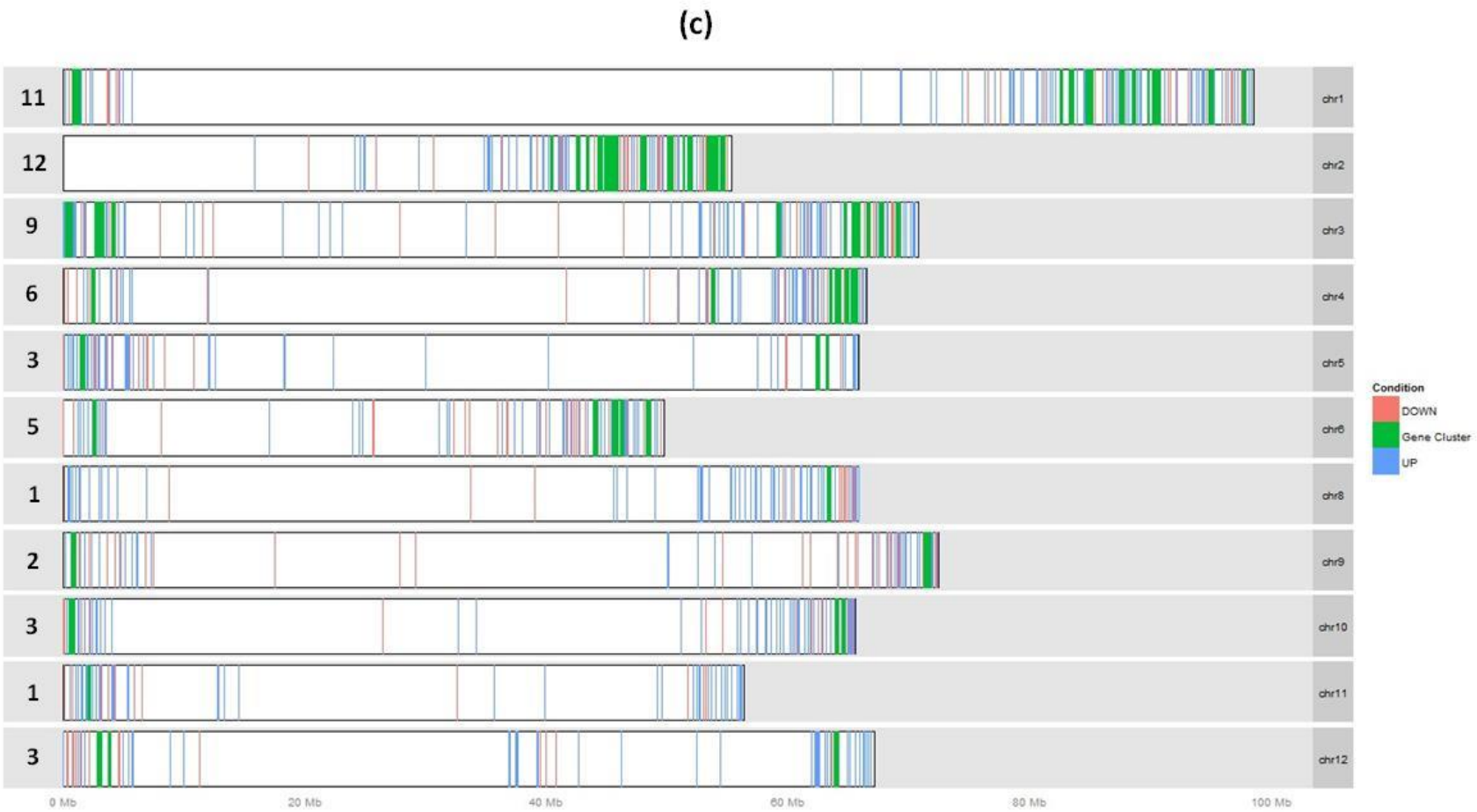

Figure 14. Panel (c), Distribution of DEGs along F1 hybrid chromosomes. Down-regulated genes in *infested vs non-infested* condition are colored in pink, while up-regulated DEGs are in light blue; the green colored portions represent gene clusters. Total number of identified gene clusters is presented on the left side of the chromosome.

4. DISCUSSION

Plants are highly complex systems, composed of densely interconnected elements, arranged in a sort of hierarchical manner from cellular level to the whole plant and ecosystem level. The properties of any complex system in connection with others can be better understood using different –omics approaches. In this framework we carried out an integrated study to investigate the molecular mechanisms underlying the interaction between tomato and the leafminer *T. absoluta*. To our knowledge this is the first study that integrates transcriptomic-, metabolomic- and genomic-based studies to investigate this kind of interaction in cultivated tomato varieties. To date, information about resistance traits to this herbivore are available only for wild tomato genotypes such as *S. habrochaites* and *S. pennellii* (de Oliveira et al., 2012; Maluf et al., 2010; Proffit et al., 2011). Three different cultivated tomato varieties were employed for the experiment: a putatively tolerant (T) line, that maintains a good level of fitness after the insect infestation and herbivores feeding; a susceptible (S) line that is highly damaged after herbivores feeding; a breeding F1 hybrid (F1) obtained crossing the above mentioned lines that showed an intermediate behavior. Integrating a RNA sequencing analysis and a NMR-based metabolomic study, we were able to investigate the response to *T. absoluta*, at two genome expression level both in the T and S genotypes andto follow inherited traits in the F1 hybrid. Furthermore, the genomic study based on the identification of structural variants helped us to investigate if the different responses to the leafminer are due to DNA nucleotidic differences among our genotypes. A quantitative analysis of differentially expressed genes (DEGs) obtained from the RNA-Seq experiment showed that the T genotype has a higher number of transcripts activated and/or inhibited after the *T. absoluta* disturbance, compared to the S genotype. The Tolerant genotype deploys a huge transcriptional reprogramming in order to respond to the herbivore feeding that turns into a complex re-arrangement of primary and secondary metabolisms. A significant switch of gene categories related to the perception of the damage and subsequent signaling activation, leading to a systemic response to the leafminer feeding, was revealed in such genotype. The response activated could be explained with the up-regulation of a serine/threonine-protein kinase receptor (Solyc05g006570.1). This protein is involved in the perception of systemin, a peptide hormone responsible for the systemic activation of defense genes in leaves of wounded plants, first isolated in tomato leaves (Pearce et al., 1991). Such peptide interacts with target cells to activate intracellular events that lead to the release of linolenic acid (LA) from cellular membranes, its conversion into octadecanoid pathway, and the subsequent activation of direct and indirect defenses to prevent the leafminer damage (Ryan 2000; Sun et al.

2011). Moreover, systemin has been shown to trigger an increase of intracellular Ca^{2+} (calmodulins) in tomato mesophyll cells (Moyen et al., 1998). Elevations in cytosolic Ca^{2+} concentrations lead to the formation of active Ca^{2+}/CaM complexes, which could modulate several cellular functions by interacting with regulatory proteins including kinases, phosphatases, lipases and ion transporters (Snedden & Fromm, 2001; Tidow & Nissen, 2013). In plants, Calmodulin (CaM), Ca^{2+}-binding proteins, play a key regulatory role in many cellular processes, including responses to external stimuli. Transgenic tomato plants that over-express the systemin gene were found to express increased levels of CaM mRNA and protein in leaves compared to wild-type plants (Bergey & Ryan, 1999). Therefore the up-regulation of transcripts related to calmodulins and calmodulin-related proteins found in the T genotype could be correlated to the systemic response mediated by systemin. The activation of octadecanoid pathway, induced by systemin, leads to the production of JA to provide the above mentioned systemic signal (Lee & Howe, 2003; Li et al., 2002; Schilmiller & Howe, 2005). Our results highlighted that JA pathway plays a pivotal role in the tomato-*T. absoluta* interaction since a strong up-regulation of genes coding for enzymes of this pathway were detected in the Tolerant genotype. JA is a signaling molecule whose activity in plant-insect interactions has been well elucidated in the last years (Erb et al., 2012; Grinberg-Yaari et al., 2015). The octadecanoid pathway for JA biosynthesis initiated in the chloroplast and terminated in peroxisomes and many of the enzymes and corresponding genes involved in the pathway have been identified (Schaller et al., 2005). JA is synthesized from alpha-linolenic acid, which is a C_{18} poly-unsaturated fatty acid. Alpha-linolenic acid is then oxidized by lipoxygenases to form 13-hydroperoxylinolenic acid, which is then modified by a dehydrase and undergoes cyclization by allene oxide cyclase to form 12-oxo-phytodienoic acid. Then, such molecule is subject to a reduction process and three rounds of beta-oxidation to form jasmonic acid (Howe, 2001). Furthermore, a catabolization step can occur to form Methyl-Jasmonate (Me-JA). Me-JA is the volatile counterpart of JA and could be a good candidate for such intra- and inter-cellular signal transducers because it can diffuse through the membranes (Jang et al., 2014). A JA-O-methyltransferase, mediating this catabolization step, resulted up-regulated in the T genotype, suggesting that Me-JA may is activated to promote the defensive gene expression during the tomato response to *T. absoluta*. The susceptible genotype showed a different behavior during the *T. absoluta* challenge. In particular, the transcription of systemin or systemin-related gene that could lead to an active recognition of the insect or a perception of the damage was not evidenced. Such finding could explain why the S genotype cannot deploy a series of responses to maintain its fitness. Indeed, the down-regulation of genes from the core pathway of JA and the lack of any

methyltransferase related to the Me-JA synthesis confirms that the defense response is not activated in the susceptible line.

The activation of recognition patterns mediated by the systemin/octadecanoid pathwaty can activate a direct and an indirect defense mechanism. In the direct mechanism, plant structural elements such as leaf surface waxes and/or trichomes work as a first physical barrier to feeding by the herbivores; furthermore, secondary metabolites act as repellents to herbivores, forming the next barriers that defend the plant from subsequent attack (Strauss et al., 2002; Hanley et al., 2007). Tolerant genotype, in this framework, showed the activation of a whole plethora of genes to directly combat the *T. absoluta* feeding. Among those, genes involved in trichomes formation and differentiation have been detected. A strong up-regulation of genes related to auxin pathway, a well-known player in plant growth and development and trichomes formation (Deng et al., 2012; Zhang et al., 2015), was detected. Particularly, AUX/IAA transcription factors, aldehyde oxidase, tryptophan synthase, IAA-amino acid hydrolases were strongly up-regulated. A particular transcription factor, *SlbHLH150* (Solyc09g065100), belonging to helix-loop-helix gene family and playing an important role in leaf physiological and developmental processes (Sun et al., 2015), resulted up-regulated in the T genotype and under-expressed in the S genotype. Trichomes play a main role in plant defense against many insect pests and involve both toxic and deterrent effects, affecting negatively the ovipositional behavior, feeding and larval nutrition of insect pests (Handley et al., 2005). In addition, glandular trichomes secrete secondary metabolites including flavonoids, terpenoids and alkaloids that can be poisonous, repellent, or trap insects and other organisms, thus forming a combination of structural and chemical defense. Integration of transcriptome and metabolome data revealed that the tolerance response to the herbivore could be due to the production and formation of Acylsugars. These allochemicals confer resistance to a large number of arthropod pests, including the tomato pinworm, *T. absoluta* (de Resende et al., 2006). These aliphatic esters of sucrose and glucose are produced normally in glandular trichomes of tomato and potato (Lawson et al., 1997; Bonierbale et al., 1994), but high concentrations of these compounds could be found in leaves of accessions of the wild tomato *Solanum pennellii*. Current tomato commercial cultivars lack high acylsugar content, whereas F1 plants from a cross between *Solanum lycopersicum* and *Solanum pennellii*showed a moderate content (Resende et al., 2002). Directly connected to the wound-responses mediated by systemins and JA signaling molecules is the activation of different Protease Inhibitors (PIs). This class resulted widely up-regulated in the T genotype in the response to the leafminer. PIs bind to the digestive enzymes in insect gut and inhibit their activity, thereby reduce protein digestion, resulting in the short-age of amino acids, and slow development and/or starvation of the insects (Azzouz et al, 2005). The NMR analysis helped us to

shed more light on direct defense mechanisms involved in this interaction. The metabolomic analysis performed on our genotypes evidenced differences in metabolites production after the herbivore feeding and, especially in the T line, interesting compounds were detected. Above all, the piridinic alkaloid Trigonelline was produced in higher amounts after the *T. absoluta* disturbance in the T genotype and in smaller amounts in S. Trigonelline (i.e. nicotinic acid betaine) is an alkaloid with multiple regulatory functions in plants, such as cell cycle, nodulation, oxidative, UV and salt stress response, DNA methylation (Minorsky, 2002). To date, no strong evidence exists about an involvement of this kind of compound in pest resistance. Only Mirnezhad and colleagues (2009) identified very low amounts of this compound in some tomato varieties resistant to *Frankliniella occidentalis*, hypothesizing that this observation may be the result of a metabolic trade-off favouring the production of acylsugars. In contrast in our work, trigonelline was produced abundantly in T genotype and therefore such finding needs further investigations. Higher concentration of GABA was also identified in our infested genotypes, with no differences among them. g-Aminobutyric acid (GABA) is a four-carbon non-protein amino acid conserved from bacteria to plants and vertebrates. Its pathway in plants (also known as 'GABA shunt') is composed of the cytosolic enzyme glutamate decarboxylase (GAD) and the mitochondrial enzymes GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH) (Bouché & Fromm, 2004). Genes coding for such enzymes were also found up-regulated in both T and S genotypes. Since GABA is a neurotransmitter in vertebrates and invertebrates, it was speculated that it could be produced by plants to deter insect feeding, hypothesizing that its ingestion interferes with the normal development of insects (Shelp et al., 1999). GABA levels are elevated by mechanical stimulation or damage (Ramputh & Bown, 1996) and even insects footsteps on leaves induces its production (Bown et al., 2002). Transgenic tobacco plants containing elevated GABA levels were resistant to root-knot nematodes (McLean et al., 2003) and tobacco budworm larvae (MacGregor et al., 2003). All this findings corroborate our assumption of a leading role of GABA in the interaction between tomato and *T. absoluta*, even if no particular differences could be detected between Tolerant and Susceptible genotypes. Combined action of mechanical damage and elicitors from the attacking herbivore induces plant responses mediated basically by volatile compounds (VOCs), synthesized in order to attract natural enemies of herbivores and, at the same time, move them away. This indirect activation of defense in our tomato genotypes is evidenced thanks to an activation of genes basically connected to production of VOCs belonging to isoprenoids and phenylpropanoid classes. Isoprenoids are a hugely diverse family of compounds derived from the C5 precursor's isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) and in plants they are specialized secondary metabolites that participates also in plant-herbivores

interactions (Vranovà et al., 2012). Among isoprenoids, terpenes are probably the largest and structurally most diverse class of plant metabolites, with a primary role in plant defense (Tholl, 2006). In particular, volatile terpenoids are the most abundant metabolites in tomato vegetative tissues and particularly in trichomes, playing a major role in resistance against herbivores; these can be classified into two groups: monoterpenoids (C10) and sesquiterpenoids (C15) (Champagne & Boutry, 2016), both synthesized from the five-carbon precursors IPP and DMAPP. The Tolerant genotype shows a whole repertoire of genes coding for different classes of terpenoids (monoterpenes, diterpenes, hemiterpenes and triterpenes) that result to be down-regulated in the Susceptible line. A (E)-Beta-ocimene synthase (Solyc01g105920.2) is expressed at very low expression also in the S line. The involvement of this volatile compound is already described in plant-insect interactions, since airborne (E)-beta-ocimene emitted from plants can serve as a chemical cue for the attraction of parasitoids or predators of plant herbivores and also as an attractant for pollinating insects (Navia-Ginèet al., 2009; Cascone et al., 2014; Proffit et al., 2011). Moreover, other terpenes were already evidenced in the interaction between tomato and *T. absoluta*, by Azevedo et al. 2003, and particularly the zingiberene was associated with a reduction in oviposition and feeding damage. Like terpenoids, also phenylpropanoids result to be key drivers of the response to *T. absoluta* since both transcriptomic and metabolomic investigations revealed their involvement. These compounds are generally mediators of the plants defense (Dixon et al., 1995; Dixon et al., 2002; La Camera et al., 2004) and, in particular, volatile phenylpropanoids produced by plant trichomes have a leading role in herbivores resistance (Glas et al., 2012). The Tolerant genotype displays a complete up-regulation of genes encoding for all the branches of phenylpropanoid core pathway as well as for subsequent reactions leading to the production of volatile phenylpropanoids. Differences between T and S occur especially for production of volatile benzenoid esters and methyl-eugenol. Genes coding for such compounds, respectively, two benzoyl transferases (Solyc07g049660; Solyc05g015800) and an eugenol-O-methyltransferase (EOM, Solyc10g008120.2), could be key mediators of the response to *T. absoluta,*, since they are strongly up-regulated only in the T line. The first two transferases catalyze for the formation of benzylbenzoate, a major volatile produced after leaf disruption and wounding. Interestingly, the most similar protein to these transferase found in *Arabidopsis* catalyzes the formation of cis-3-hexen-1-ol acetate, another green-leaf volatile using acetyl CoA as substrate (D'Auria et al., 2002). EOM is an enzyme catalyzing the biosynthesis of Methyl Eugenol (ME) in different plant species; it is directly derived from eugenol, a product from phenylalanine (an essential amino acid) through caffeic acid and ferulic acid via the shikimate pathway. Toxicity of ME was demonstrated by Bhardwaj et al. (2010) against larvae of the tobacco armyworm, *Spodoptera litura*. Metabolome

analysis revealed also a high content of compounds such as Chlorogenic, neo-chlorogenic and Feruloyl-quinic acids that have negative effect on caterpillars (Bernays et al., 2000; Beninger et al., 2004) as well as for different leaf beetles (Fulcher et al., 1998; Ikonen, et al., 2001; Jassbi, 2003) and for thrips in chrisantemum (Leiss et al., 2009). Methyl-salicylate (MeSA), a volatile compound released by herbivore attacked plants, could be involved also in *T. absoluta* response since SAMT (S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase 1, Solyc01g081340.2), the key gene of the pathway resulted strongly up-regulated only in the T genotype. Methyl-salicylate displays its activity both as repellent for herbivores and attractant for parasitoids (Zhu & Park, 2005). Correlation between methyl-salicylate production and JA signaling upon herbivory has been assessed in tomato mutant *def-1*, which is deficient in induced jasmonic acid accumulation upon wounding or herbivory. The expression of the MeSA biosynthetic gene salicylic acid methyltransferase (SAMT) was induced by spider mite (*Tetranichus urticae*) infestation in wild type but not in *def-1* (Ament et al., 2004). As mentioned in the result section of this manuscript, different genes result to be in common with the tolerant genotype and the F1 hybrid. In particular, we could detect some key genes involved in the response to *T. absoluta* such as those involved in JA biosynthesis (FAD2 and ACX), monoterpene's (E)-Beta-ocimene synthases (Solyc01g105890.2 and Solyc01g105920.2) and volatiles phenylpropanoid coding genes, that we deeply discussed above. Though inheritance of those traits is not particularly strong, they assure to the plant a sort of 'basal' tolerance, in order to avoid the herbivore feeding.
We took advantage of high amount of sequences obtained by RNA-Seq technique for identifying sequence polymorphism in differentially expressed genes associated with a trait of interest for resistance in *T. absoluta* and to find traits useful for tomato crop breeding. Mapping the reads of T, S and F1genotypes against the *S. lycopersicum* reference genome, we were able to detect transcriptomic variants, in particular SNPs and little Insertions and Deletions. A high number of SNPs and InDels was detected in our genotypes. Variation in the level of polymorphism among chromosomes was found. A high number of variants were detected on chromosomes 01 and 07 (around 41000 and 36000 respectively for each sample). Sim and colleagues (2012) genotyping a collection of 426 tomato accessions, using the SolCAP SNP array, revealed that some genomic regions can have higher genetic variation between sub-populations, suggesting that historical breeding practices have led to different patterns of genetic variation in cultivated tomato germplasm.

To get more insights of the mean of gene variants occurring between T, S and F1 genotypes and the reference genome, we analyzed genes putatively involved in the response to *T. absoluta* and whose role has been already discussed. High percent of variation and deleterious substitutions has been

found in genes belonging to secondary metabolism and hormones classes. In particular, genes encoding terpene biosynthetic pathways were shown to be affected by deleterious variants in the T genotype compared to the reference genome. A deeper investigation of variants affecting genes involved in the herbivore response, revealed that the great majority of those genes in S genotype are affected by substitutions that lead mainly to stop codons, subsequently blocking the final protein synthesis. The E-beta-ocimene synthase (Solyc01g105920.2) shared by both T and S genotypes, reveled a stop gained mutation in S as well as genes involved in the phenylpropanoid pathway, in the cytochrome p450 complex and lipoxygenases related to JA production. This furthermore confirms that phenotypic differences observed in T and S genotypes are highly influenced by nucleotidic changes. Interestingly, genes shared between T and F1 hybrid, showed variations, in the same positions. The chromosome , distribution of variants permitted us to detect variant-peak regions. This allowed to identify chromosome candidate regions containing genes putatively involved in *T. absoluta* response that could be used in future molecular breeding efforts. On chromosome 01, a peak including two genes involved in the acylsugars production (SlAT1 and SlAT2) were detected in a region at 93 Mb. In particular SlAT2, is a member of the BAHD family of acyltransferases that was shown to encode an acetyl-CoA–dependent acyltransferase enzyme capable of acetylation of these allochemicals in vitro. RNAi suppression of SlAT2 in transgenic *S. lycopersicum* cv. M82 resulted in reduced acylsugar acetylation (Schilmiller et al., 2012). Genes for aldose-1-epimerase-like, an enzyme involved in conversion of alpha-glucose to beta-glucose, and different Acyl-transferases, were also identified in peak regions of chromosome 02. Analysis of variant-peaks revealed interesting results also on chromosomes 07, where six wound induced proteins and a SAM dependent carboxyl methyltransferase (involved in MeSa biosynthesis and discussed above), were identified. We were also able to identify how genes are arranged along tomato chromosomes and to investigate gene functional clustering (set of two or more non-homologous genes encoding enzymes from the same pathway). The Tolerant line showed the highest number and the most interesting clusters. Genes that tend to be grouped together belongs particularly to terpene biosynthetic pathway. On chromosome 8 the gene cluster (504959- 586720) in the T line includes three up-regulated terpenoid synthases (TPSs), a di-trans-poly-cis-decaprenyl-cis-transferase-like and a cytochrome p450 gene. The function of this particular cluster was described for the first time in *S. lycopersicum* by Matsuba et al. (2013); the TPSs promote the synthesis of monoterpenes and diterpenes from cis-prenyldiphosphates, substrates that are synthesized by enzymes encoded by cis-prenyltransferase (CPT), also located within the same cluster. The monoterpene synthase genes in the cluster likely evolved from a diterpene synthase gene in the cluster by duplication and divergence. In the orthologous cluster in *S. habrochaites*, a

new sesquiterpene synthase gene was created by a duplication event of a monoterpene synthase followed by a localized gene conversion event directed by a diterpene synthase gene. Other interesting clusters were furthermore identified on chromosome 8 of the T genotype for genes related to phenylpropanoids volatile compounds.

5. CONCLUSIONS

Our multi-omic study proved to be very useful for identifying molecular mechanisms involved in tomato response to *T. absoluta*.

In the Tolerant genotype, the reprogramming driven by both direct and indirect defenses is based on an antixenosis mechanism. This is characterized by the lower utilization of the host by the herbivore due to chemicals, physical and morphological barriers. The RNA-Seq gene expression analysis allowed us to assess an active recognition of the insect that leads to a signaling cascade mediated by the systemin/jasmonic acid complex and, subsequently, the activation of genes involved in the growth of trichomes (physical barriers) together with the activation of genes coding for production of volatile terpenes and phenylpropanoids. A direct defense has been well elucidated by the metabolome analysis, revealing an involvement of compounds such as chlorogenic and neo-chlorogenic acids, GABA and, pyridinc alkaloid trigonelline.

The susceptible genotype demonstrates to be less capable of deploying a real defensearsenal. Down-regulation of key genes in JA pathway and absence of genes differentially expressed in key pathways such as terpenes and phenylpropanoids have been identified. T line showed a high level of variations on above mentioned genes belonging to the secondary metabolism compartment and, genes of the same compartment in the S line, showed some deleterious variants (such as stop codons) that lead to a stop of final protein synthesis.

The F1 derived from the cross between the Tolerant and Susceptible lines expresses, even if at less extent, the key genes identified in the tolerant line, as well as the same structural polymorphisms. Such finding is of great importance since it is a commercialized variety that showed good agronomic performance and tolerance to the leafminer*T.absoluta*. Indeed, the information gathered in this study could be very useful for better direct future tomato breeding.

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SUPPLEMENTARY MATERIALS

Table S1. Number of sequenced reads before and after quality controls.

Sample Name	Number of reads before data quality control	Number of reads after data quality control
F1_C1	20633578	19582087
F1_C2	22164922	21505973
T_C1	25713821	24390437
T_C2	19706221	19069911
S_C1	22224662	21391274
S_C2	23663488	22993345
F1_I1	20985515	20239883
F1_I2	26810257	25557614
F1_I3	27554483	26449214
T_I1	28569521	27162027
T_I2	28968237	27930286
T_I3	30377806	28976601
S_I1	22486053	21458005
S_I2	24132828	22930279
S_I3	27851400	26308810

Table S2. Gene Ontology categories obtained after the GO enrichment analysis, classified by Genotype (if unique or common) and by domain (cellular component, molecular function, biological process).

Genotype	GO Domain	GO ID	GO Term	DEGs	FDR
T unique	Cellular Component	GO:0030660	Golgi-associated vesicle membrane	8	0,038
		GO:0009295	nucleoid	13	0,0075
		GO:0042646	plastid nucleoid	10	0,038
		GO:0009532	plastid stroma	125	4,3E-10
		GO:0009570	chloroplast stroma	117	6,9E-09
		GO:0005768	endosome	46	0,02
		GO:0009941	chloroplast envelope	109	0,000035
		GO:0009526	plastid envelope	110	0,000082
		GO:0048046	apoplast	62	0,012
		GO:0044435	plastid part	214	1,1E-07
		GO:0044434	chloroplast part	209	2,2E-07
		GO:0005794	Golgi apparatus	118	0,00039
		GO:0005886	plasma membrane	357	4,2E-10
		GO:0005773	vacuole	138	0,00018
		GO:0044436	thylakoid part	65	0,036
		GO:0044459	plasma membrane part	160	0,000074
		GO:0005774	vacuolar membrane	82	0,012
		GO:0044437	vacuolar part	82	0,012
		GO:0031984	organelle subcompartment	75	0,029
		GO:0009506	plasmodesma	140	0,00064
		GO:0055044	symplast	140	0,00064
		GO:0005911	cell-cell junction	140	0,00065
		GO:0030054	cell junction	140	0,00065
		GO:0009534	chloroplast thylakoid	73	0,036
		GO:0031976	plastid thylakoid	73	0,038
		GO:0031967	organelle envelope	146	0,00072
		GO:0031975	envelope	147	0,00087
		GO:0009507	chloroplast	338	2,4E-07
		GO:0009536	plastid	351	2,4E-07
		GO:0009579	thylakoid	101	0,042
		GO:0005829	cytosol	230	0,00042
		GO:0005737	cytoplasm	964	1,4E-10
		GO:0044444	cytoplasmic part	883	1,5E-09
		GO:0031090	organelle membrane	182	0,027
		GO:0044422	organelle part	517	0,000039

	GO:0043227	membrane-bounded organelle	1008	1,7E-08
	GO:0044446	intracellular organelle part	516	0,000045
	GO:0043231	intracellular membrane-bounded organelle	1003	2,5E-08
	GO:0016020	membrane	855	4,4E-06
	GO:0044424	intracellular part	1296	2,4E-07
	GO:0005622	intracellular	1317	4,2E-07
	GO:0043226	organelle	1077	7,5E-06
	GO:0043229	intracellular organelle	1075	8,2E-06
	GO:0005623	cell	1780	2,5E-07
	GO:0044464	cell part	1780	2,5E-07
	GO:0044425	membrane part	495	0,011
Molecular Function	GO:0016810	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	25	0,035
	GO:0016881	acid-amino acid ligase activity	70	0,00043
	GO:0016874	ligase activity	109	4,6E-06
	GO:0016879	ligase activity, forming carbon-nitrogen bonds	82	0,00043
Biological Process	GO:0006914	autophagy	14	0,00042
	GO:0009226	nucleotide-sugar biosynthetic process	7	0,049
	GO:0009225	nucleotide-sugar metabolic process	11	0,031
	GO:0035195	gene silencing by miRNA	11	0,044
	GO:0019395	fatty acid oxidation	12	0,049
	GO:0009626	plant-type hypersensitive response	14	0,045
	GO:0048764	trichoblast maturation	19	0,029
	GO:0048765	root hair cell differentiation	19	0,029
	GO:0048469	cell maturation	19	0,035
	GO:0010054	trichoblast differentiation	20	0,029
	GO:0033014	tetrapyrrole biosynthetic process	20	0,029
	GO:0006779	porphyrin biosynthetic process	18	0,049
	GO:0035194	posttranscriptional gene silencing by RNA	18	0,049
	GO:0010053	root epidermal cell differentiation	23	0,022
	GO:0016054	organic acid catabolic process	26	0,024
	GO:0046395	carboxylic acid catabolic process	26	0,024
	GO:0033013	tetrapyrrole metabolic process	25	0,031
	GO:0006778	porphyrin metabolic process	24	0,038
	GO:0000302	response to reactive oxygen species	25	0,047
	GO:0009642	response to light intensity	27	0,044
	GO:0009620	response to fungus	49	0,0053
	GO:0009657	plastid organization	41	0,024

GO:0031324	negative regulation of cellular metabolic process	39	0,045
GO:0044271	cellular nitrogen compound biosynthetic process	86	0,00026
GO:0048523	negative regulation of cellular process	65	0,0035
GO:0007154	cell communication	85	0,00044
GO:0016567	protein ubiquitination	42	0,05
GO:0006631	fatty acid metabolic process	60	0,0094
GO:0009892	negative regulation of metabolic process	54	0,025
GO:0051186	cofactor metabolic process	84	0,0022
GO:0032787	monocarboxylic acid metabolic process	93	0,0012
GO:0009617	response to bacterium	55	0,035
GO:0051707	response to other organism	119	0,00026
GO:0044282	small molecule catabolic process	61	0,028
GO:0010035	response to inorganic substance	110	0,00043
GO:0048519	negative regulation of biological process	93	0,0022
GO:0044106	cellular amine metabolic process	112	0,00042
GO:0006082	organic acid metabolic process	196	4,4E-07
GO:0019752	carboxylic acid metabolic process	195	4,4E-07
GO:0043436	oxoacid metabolic process	195	4,4E-07
GO:0009605	response to external stimulus	83	0,0056
GO:0042180	cellular ketone metabolic process	199	4,4E-07
GO:0006520	cellular amino acid metabolic process	101	0,0022
GO:0009308	amine metabolic process	122	0,0012
GO:0071310	cellular response to organic substance	81	0,022
GO:0051704	multi-organism process	158	0,00026
GO:0006970	response to osmotic stress	87	0,02
GO:0010038	response to metal ion	77	0,044
GO:0015031	protein transport	89	0,024
GO:0045184	establishment of protein localization	89	0,024
GO:0008104	protein localization	96	0,02
GO:0009607	response to biotic stimulus	128	0,0036
GO:0007242	intracellular signaling cascade	111	0,0094
GO:0016053	organic acid biosynthetic process	87	0,049
GO:0046394	carboxylic acid biosynthetic process	87	0,049
GO:0044283	small molecule biosynthetic process	140	0,0042
GO:0023052	signaling	206	0,00026
GO:0044248	cellular catabolic process	213	0,00037
GO:0009628	response to abiotic stimulus	231	0,00026

		GO:0009653	anatomical structure morphogenesis	108	0,035
		GO:0023046	signaling process	161	0,0037
		GO:0023060	signal transmission	160	0,0045
		GO:0048513	organ development	142	0,012
		GO:0048731	system development	142	0,012
		GO:0006519	cellular amino acid and derivative metabolic process	122	0,029
		GO:0007165	signal transduction	155	0,012
		GO:0009056	catabolic process	230	0,0027
		GO:0044281	small molecule metabolic process	431	0,000015
		GO:0006996	organelle organization	171	0,044
		GO:0010033	response to organic substance	173	0,045
		GO:0043412	macromolecule modification	397	0,00042
		GO:0006464	protein modification process	365	0,0022
		GO:0043687	post-translational protein modification	339	0,0038
		GO:0048856	anatomical structure development	228	0,044
		GO:0042221	response to chemical stimulus	311	0,024
		GO:0032502	developmental process	284	0,035
		GO:0006793	phosphorus metabolic process	314	0,049
		GO:0050896	response to stimulus	566	0,0056
		GO:0051179	localization	395	0,045
		GO:0009987	cellular process	1794	0,0048
S unique	Molecular Function	GO:0016758	transferase activity, transferring hexosyl groups	28	0,0096
		GO:0042555	MCM complex	6	7,3E-08
		GO:0042646	plastid nucleoid	5	0,0021
		GO:0009295	nucleoid	6	0,00097
		GO:0046658	anchored to plasma membrane	7	0,0008
		GO:0031226	intrinsic to plasma membrane	7	0,002
		GO:0009505	plant-type cell wall	22	1E-08
		GO:0044454	nuclear chromosome part	6	0,0058
T/S/F1 common	Cellular Component	GO:0031225	anchored to membrane	7	0,0036
		GO:0000228	nuclear chromosome	6	0,021
		GO:0048046	apoplast	25	2,9E-07
		GO:0005618	cell wall	43	1,5E-10
		GO:0030312	external encapsulating structure	43	1,5E-10
		GO:0009532	plastid stroma	31	3,4E-06
		GO:0005730	nucleolus	17	0,0011
		GO:0009570	chloroplast stroma	28	0,000033

GO:0005576	extracellular region	32	0,000011
GO:0044435	plastid part	57	2,2E-08
GO:0044434	chloroplast part	54	1,2E-07
GO:0044437	vacuolar part	22	0,0013
GO:0005774	vacuolar membrane	22	0,0013
GO:0042651	thylakoid membrane	15	0,01
GO:0031981	nuclear lumen	24	0,0013
GO:0009534	chloroplast thylakoid	19	0,0046
GO:0031976	plastid thylakoid	19	0,0047
GO:0005694	chromosome	14	0,022
GO:0009535	chloroplast thylakoid membrane	13	0,031
GO:0031984	organelle subcompartment	19	0,0057
GO:0070013	intracellular organelle lumen	28	0,0008
GO:0043233	organelle lumen	28	0,0008
GO:0055035	plastid thylakoid membrane	13	0,032
GO:0044436	thylakoid part	16	0,015
GO:0031974	membrane-enclosed lumen	28	0,0009
GO:0009941	chloroplast envelope	24	0,0023
GO:0009507	chloroplast	85	9,2E-09
GO:0009526	plastid envelope	24	0,0034
GO:0005773	vacuole	32	0,0008
GO:0009536	plastid	86	2E-08
GO:0009579	thylakoid	25	0,0038
GO:0055044	symplast	32	0,0013
GO:0009506	plasmodesma	32	0,0013
GO:0005911	cell-cell junction	32	0,0013
GO:0030054	cell junction	32	0,0013
GO:0044459	plasma membrane part	34	0,0019
GO:0031090	organelle membrane	44	0,00062
GO:0005739	mitochondrion	25	0,016
GO:0044446	intracellular organelle part	120	7,3E-08
GO:0044422	organelle part	120	7,3E-08
GO:0043232	intracellular non-membrane-bounded organelle	53	0,00055
GO:0043228	non-membrane-bounded organelle	53	0,00055
GO:0044428	nuclear part	25	0,024
GO:0005886	plasma membrane	61	0,0031
GO:0044444	cytoplasmic part	168	6,8E-06

		GO:0005737	cytoplasm	178	0,000011
		GO:0043227	membrane-bounded organelle	178	0,00051
		GO:0043231	intracellular membrane-bounded organelle	177	0,00055
		GO:0043229	intracellular organelle	197	0,00042
		GO:0043226	organelle	197	0,00042
		GO:0005622	intracellular	227	0,0013
		GO:0044424	intracellular part	218	0,0032
		GO:0044464	cell part	302	0,0013
		GO:0005623	cell	302	0,0013
		GO:0016020	membrane	138	0,04
	Molecular Function	GO:0016628	oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor	5	0,031
		GO:0016762	xyloglucan:xyloglucosyl transferase activity	7	0,016
		GO:0005507	copper ion binding	17	0,021
	Biological Process	GO:0006270	DNA replication initiation	7	0,000015
		GO:0006261	DNA-dependent DNA replication	8	0,011
		GO:0006364	rRNA processing	8	0,04
		GO:0042254	ribosome biogenesis	12	0,0053
		GO:0009658	chloroplast organization	10	0,038
		GO:0022613	ribonucleoprotein complex biogenesis	13	0,011
		GO:0009657	plastid organization	13	0,026
		GO:0006260	DNA replication	12	0,04
T/F1 common	Cellular Component	GO:0005618	cell wall	24	0,0016
		GO:0005794	Golgi apparatus	22	0,015
		GO:0005802	trans-Golgi network	12	0,012
		GO:0005874	microtubule	8	0,047
		GO:0005886	plasma membrane	48	0,022
		GO:0016020	membrane	116	0,02
		GO:0016021	integral to membrane	51	0,047
		GO:0030312	external encapsulating structure	24	0,0016
		GO:0031224	intrinsic to membrane	52	0,047
		GO:0044425	membrane part	71	0,048
		GO:0044430	cytoskeletal part	12	0,03
		GO:0044431	Golgi apparatus part	13	0,03
	Molecular Function	GO:0016627	oxidoreductase activity, acting on the CH-CH group of donors	7	0,029
		GO:0016854	racemase and epimerase activity	5	0,032
		GO:0016857	racemase and epimerase activity, acting on carbohydrates and derivatives	5	0,029
	Biological	GO:0005975	carbohydrate metabolic process	42	0,0022

	Process	GO:0006073	cellular glucan metabolic process	12	0,012
		GO:0009765	photosynthesis, light harvesting	11	3,3E-07
		GO:0016051	carbohydrate biosynthetic process	14	0,03
		GO:0019684	photosynthesis, light reaction	11	0,028
		GO:0034637	cellular carbohydrate biosynthetic process	14	0,016
		GO:0044042	glucan metabolic process	12	0,012
		GO:0044262	cellular carbohydrate metabolic process	26	0,016
		GO:0044264	cellular polysaccharide metabolic process	15	0,012
T/S common	Cellular Component	GO:0005853	eukaryotic translation elongation factor 1 complex	5	0,0016
		GO:0022625	cytosolic large ribosomal subunit	54	6,4E-37
		GO:0022627	cytosolic small ribosomal subunit	36	1,5E-23
		GO:0016282	eukaryotic 43S preinitiation complex	13	2E-08
		GO:0033290	eukaryotic 48S preinitiation complex	13	2E-08
		GO:0070993	translation preinitiation complex	13	2E-08
		GO:0015934	large ribosomal subunit	67	8,9E-41
		GO:0033279	ribosomal subunit	115	7,7E-65
		GO:0044445	cytosolic part	97	1,2E-54
		GO:0005852	eukaryotic translation initiation factor 3 complex	16	2,6E-09
		GO:0030684	preribosome	6	0,002
		GO:0022626	cytosolic ribosome	109	3,1E-56
		GO:0015935	small ribosomal subunit	48	7,2E-25
		GO:0032040	small-subunit processome	5	0,01
		GO:0005759	mitochondrial matrix	13	0,000016
		GO:0031980	mitochondrial lumen	13	0,000016
		GO:0005840	ribosome	262	2,1E-99
		GO:0030529	ribonucleoprotein complex	296	2,8E-104
		GO:0005730	nucleolus	105	5,4E-35
		GO:0005741	mitochondrial outer membrane	10	0,0074
		GO:0005753	mitochondrial proton-transporting ATP synthase complex	7	0,042
		GO:0010319	stromule	10	0,018
		GO:0031968	organelle outer membrane	14	0,0033
		GO:0043228	non-membrane-bounded organelle	370	2,5E-66
		GO:0043232	intracellular non-membrane-bounded organelle	370	2,5E-66
		GO:0031974	membrane-enclosed lumen	151	1,8E-26
		GO:0043233	organelle lumen	149	4E-26
		GO:0070013	intracellular organelle lumen	149	4E-26
		GO:0009570	chloroplast stroma	120	1E-20

GO:0019867	outer membrane	15	0,0054
GO:0031977	thylakoid lumen	14	0,0087
GO:0031981	nuclear lumen	120	9E-20
GO:0009532	plastid stroma	121	1,3E-19
GO:0009526	plastid envelope	122	1,3E-17
GO:0005739	mitochondrion	145	3,7E-20
GO:0031969	chloroplast membrane	13	0,03
GO:0009941	chloroplast envelope	114	2,6E-15
GO:0005829	cytosol	285	8,7E-35
GO:0044429	mitochondrial part	50	7,9E-07
GO:0044428	nuclear part	143	7,9E-18
GO:0031967	organelle envelope	167	4,3E-20
GO:0042170	plastid membrane	13	0,05
GO:0031975	envelope	167	1,5E-19
GO:0044434	chloroplast part	219	7,2E-25
GO:0044435	plastid part	222	7,2E-25
GO:0005774	vacuolar membrane	87	9,1E-10
GO:0044437	vacuolar part	87	9,1E-10
GO:0009536	plastid	382	6E-36
GO:0009507	chloroplast	362	1,7E-33
GO:0032991	macromolecular complex	435	3,4E-39
GO:0005740	mitochondrial envelope	38	0,00079
GO:0000786	nucleosome	21	0,025
GO:0031966	mitochondrial membrane	34	0,0024
GO:0044446	intracellular organelle part	572	1,1E-40
GO:0044422	organelle part	572	1,1E-40
GO:0044444	cytoplasmic part	940	5,2E-59
GO:0032993	protein-DNA complex	21	0,04
GO:0019866	organelle inner membrane	33	0,0064
GO:0009506	plasmodesma	131	9,9E-10
GO:0055044	symplast	131	9,9E-10
GO:0005911	cell-cell junction	131	1,1E-09
GO:0030054	cell junction	131	1,1E-09
GO:0005737	cytoplasm	987	7,6E-56
GO:0005743	mitochondrial inner membrane	25	0,036
GO:0005773	vacuole	120	6,8E-08
GO:0031090	organelle membrane	182	4,8E-11

	GO:0044459	plasma membrane part	139	1,2E-08
	GO:0031976	plastid thylakoid	63	0,00078
	GO:0031984	organelle subcompartment	64	0,00076
	GO:0009534	chloroplast thylakoid	62	0,0012
	GO:0055035	plastid thylakoid membrane	43	0,013
	GO:0043229	intracellular organelle	1060	7,5E-38
	GO:0043226	organelle	1060	1E-37
	GO:0009535	chloroplast thylakoid membrane	42	0,021
	GO:0042651	thylakoid membrane	45	0,019
	GO:0044436	thylakoid part	51	0,015
	GO:0005618	cell wall	83	0,00089
	GO:0044424	intracellular part	1218	1,7E-35
	GO:0005622	intracellular	1232	2,7E-34
	GO:0030312	external encapsulating structure	83	0,0018
	GO:0043231	intracellular membrane-bounded organelle	874	9E-23
	GO:0043227	membrane-bounded organelle	876	9E-23
	GO:0009579	thylakoid	80	0,0094
	GO:0005634	nucleus	356	3E-08
	GO:0005623	cell	1465	4,1E-17
	GO:0044464	cell part	1465	4,1E-17
	GO:0005886	plasma membrane	235	0,00047
	GO:0016020	membrane	587	0,031
Molecular Function	GO:0031369	translation initiation factor binding	5	0,01
	GO:0004576	oligosaccharyl transferase activity	6	0,0056
	GO:0008536	Ran GTPase binding	9	0,0007
	GO:0003746	translation elongation factor activity	17	1,5E-07
	GO:0003735	structural constituent of ribosome	238	5,6E-94
	GO:0017016	Ras GTPase binding	9	0,0046
	GO:0031267	small GTPase binding	9	0,0046
	GO:0005198	structural molecule activity	254	1,2E-87
	GO:0008135	translation factor activity, nucleic acid binding	44	2,3E-13
	GO:0051020	GTPase binding	9	0,036
	GO:0003743	translation initiation factor activity	26	1,3E-06
	GO:0016776	phosphotransferase activity, phosphate group as acceptor	10	0,024
	GO:0004812	aminoacyl-tRNA ligase activity	21	0,00011
	GO:0016875	ligase activity, forming carbon-oxygen bonds	21	0,00011
	GO:0016876	ligase activity, forming aminoacyl-tRNA and related compounds	21	0,00011

	GO:0019843	rRNA binding	23	0,000062
	GO:0019205	nucleobase, nucleoside, nucleotide kinase activity	14	0,0056
	GO:0051082	unfolded protein binding	24	0,00021
	GO:0015035	protein disulfide oxidoreductase activity	28	0,0046
	GO:0003723	RNA binding	161	1,6E-18
	GO:0015036	disulfide oxidoreductase activity	28	0,0089
	GO:0016667	oxidoreductase activity, acting on sulfur group of donors	39	0,0014
	GO:0016830	carbon-carbon lyase activity	26	0,024
	GO:0016874	ligase activity	71	0,011
Biological Process	GO:0001731	formation of translation preinitiation complex	13	3,1E-07
	GO:0006446	regulation of translational initiation	13	8,6E-07
	GO:0042026	protein refolding	7	0,0026
	GO:0009132	nucleoside diphosphate metabolic process	6	0,043
	GO:0045037	protein import into chloroplast stroma	6	0,043
	GO:0046939	nucleotide phosphorylation	9	0,0041
	GO:0006414	translational elongation	26	2,1E-09
	GO:0022618	ribonucleoprotein complex assembly	19	1,5E-06
	GO:0006412	translation	312	4,8E-106
	GO:0022613	ribonucleoprotein complex biogenesis	61	2,4E-20
	GO:0042254	ribosome biogenesis	45	3,4E-14
	GO:0045036	protein targeting to chloroplast	9	0,016
	GO:0044070	regulation of anion transport	8	0,034
	GO:0006787	porphyrin catabolic process	8	0,045
	GO:0010476	gibberellin mediated signaling pathway	8	0,045
	GO:0033015	tetrapyrrole catabolic process	8	0,045
	GO:0006413	translational initiation	27	6,8E-07
	GO:0006364	rRNA processing	25	3,7E-06
	GO:0006417	regulation of translation	23	0,000012
	GO:0016072	rRNA metabolic process	26	3,4E-06
	GO:0043269	regulation of ion transport	13	0,0061
	GO:0006418	tRNA aminoacylation for protein translation	21	0,000077
	GO:0043038	amino acid activation	21	0,000077
	GO:0043039	tRNA aminoacylation	21	0,000077
	GO:0051187	cofactor catabolic process	22	0,00023
	GO:0018193	peptidyl-amino acid modification	12	0,029
	GO:0032268	regulation of cellular protein metabolic process	28	0,000031
	GO:0017038	protein import	20	0,0013

GO:0009812	flavonoid metabolic process	17	0,0054
GO:0009813	flavonoid biosynthetic process	13	0,036
GO:0051049	regulation of transport	13	0,036
GO:0043648	dicarboxylic acid metabolic process	22	0,0011
GO:0009109	coenzyme catabolic process	14	0,031
GO:0006099	tricarboxylic acid cycle	13	0,043
GO:0046356	acetyl-CoA catabolic process	13	0,043
GO:0071478	cellular response to radiation	16	0,016
GO:0071482	cellular response to light stimulus	16	0,016
GO:0071214	cellular response to abiotic stimulus	20	0,0045
GO:0051246	regulation of protein metabolic process	28	0,00052
GO:0009637	response to blue light	17	0,024
GO:0006084	acetyl-CoA metabolic process	16	0,041
GO:0006457	protein folding	46	3,2E-06
GO:0033013	tetrapyrrole metabolic process	21	0,011
GO:0006778	porphyrin metabolic process	20	0,017
GO:0042440	pigment metabolic process	32	0,00071
GO:0008652	cellular amino acid biosynthetic process	44	0,000028
GO:0046686	response to cadmium ion	68	1,7E-07
GO:0009553	embryo sac development	23	0,018
GO:0009309	amine biosynthetic process	47	0,000035
GO:0006333	chromatin assembly or disassembly	29	0,0057
GO:0009658	chloroplast organization	23	0,036
GO:0006520	cellular amino acid metabolic process	99	1,7E-09
GO:0034622	cellular macromolecular complex assembly	70	9,4E-07
GO:0034621	cellular macromolecular complex subunit organization	79	1,8E-07
GO:0006820	anion transport	25	0,029
GO:0010608	posttranscriptional regulation of gene expression	29	0,013
GO:0009260	ribonucleotide biosynthetic process	23	0,043
GO:0031497	chromatin assembly	26	0,027
GO:0034660	ncRNA metabolic process	60	0,000016
GO:0009451	RNA modification	23	0,047
GO:0006334	nucleosome assembly	25	0,036
GO:0034728	nucleosome organization	25	0,036
GO:0044106	cellular amine metabolic process	104	4,4E-09
GO:0065004	protein-DNA complex assembly	26	0,033
GO:0044271	cellular nitrogen compound biosynthetic process	72	4,4E-06

GO:0045454	cell redox homeostasis	33	0,011
GO:0065003	macromolecular complex assembly	83	7,1E-07
GO:0043933	macromolecular complex subunit organization	92	1,5E-07
GO:0010038	response to metal ion	75	3,3E-06
GO:0009657	plastid organization	33	0,012
GO:0009991	response to extracellular stimulus	26	0,047
GO:0018130	heterocycle biosynthetic process	29	0,033
GO:0009308	amine metabolic process	113	1,1E-08
GO:0006006	glucose metabolic process	31	0,027
GO:0006519	cellular amino acid and derivative metabolic process	123	3,7E-09
GO:0044085	cellular component biogenesis	136	1E-09
GO:0009314	response to radiation	86	0,000008
GO:0009416	response to light stimulus	83	0,000012
GO:0009409	response to cold	46	0,0064
GO:0010035	response to inorganic substance	91	9,7E-06
GO:0019725	cellular homeostasis	40	0,023
GO:0051186	cofactor metabolic process	67	0,00058
GO:0044267	cellular protein metabolic process	546	1,2E-26
GO:0009059	macromolecule biosynthetic process	502	8,4E-25
GO:0022607	cellular component assembly	94	0,000053
GO:0034645	cellular macromolecule biosynthetic process	498	1,8E-24
GO:0009266	response to temperature stimulus	68	0,0025
GO:0009628	response to abiotic stimulus	200	3,3E-09
GO:0010467	gene expression	520	1,7E-22
GO:0042180	cellular ketone metabolic process	153	6E-07
GO:0044249	cellular biosynthetic process	664	6,9E-27
GO:0019752	carboxylic acid metabolic process	149	9,4E-07
GO:0043436	oxoacid metabolic process	149	9,4E-07
GO:0006082	organic acid metabolic process	149	0,000001
GO:0009651	response to salt stress	64	0,0072
GO:0006970	response to osmotic stress	69	0,0062
GO:0009058	biosynthetic process	682	4,2E-25
GO:0009605	response to external stimulus	62	0,016
GO:0019538	protein metabolic process	602	5,4E-18
GO:0016053	organic acid biosynthetic process	67	0,043
GO:0046394	carboxylic acid biosynthetic process	67	0,043
GO:0044281	small molecule metabolic process	349	6,6E-09

		GO:0044283	small molecule biosynthetic process	104	0,013
		GO:0009117	nucleotide metabolic process	85	0,046
		GO:0006753	nucleoside phosphate metabolic process	85	0,05
		GO:0042221	response to chemical stimulus	262	7,1E-06
		GO:0046483	heterocycle metabolic process	107	0,027
		GO:0044260	cellular macromolecule metabolic process	846	1,3E-09
		GO:0016043	cellular component organization	190	0,027
		GO:0050896	response to stimulus	443	0,00011
		GO:0044237	cellular metabolic process	1162	1,1E-09
		GO:0007275	multicellular organismal development	200	0,047
		GO:0032501	multicellular organismal process	216	0,043
		GO:0043170	macromolecule metabolic process	916	9,3E-07
		GO:0034641	cellular nitrogen compound metabolic process	387	0,0051
		GO:0009987	cellular process	1436	4,5E-08
		GO:0044238	primary metabolic process	1166	5,5E-06
		GO:0008152	metabolic process	1469	0,0018
S/F1 common	Molecular Function	GO:0016758	transferase activity, transferring hexosyl groups	5	0,011
		GO:0016757	transferase activity, transferring glycosyl groups	6	0,0093
	Biological Process	GO:0044262	cellular carbohydrate metabolic process	6	0,031

Table S3. Genes differentially expressed involved in the response to *Tuta absoluta* affected by deleterious variants in Tolerant genotype.

GENE	POSITION	ANNOTATION	VARIANT TYPE	AA CHANGE
Cytochrome p450				
Solyc01g080900.2	80240816	CYP88A4/ent-kaurenoate oxidase	missense_variant	p.Ser24Leu/c.71C>T
	80243158		missense_variant	p.Ser181Asn/c.542G>A
	80243634		missense_variant	p.Leu300Pro/c.899T>C
Solyc01g108210.2	95571953	CYP707A4/(+)-abscisic acid 8-hydroxylase	missense_variant	p.Ala274Thr/c.820G>A
Solyc04g011690.2	4164656	CYP75B1/flavonoid 3-monooxygenase	missense_variant	p.Asp304Asn/c.910G>A
Solyc04g071780.2	58776839	CYP75B1/flavonoid 3-monooxygenase	missense_variant	p.Arg129Lys/c.386G>A
	58776889		missense_variant	p.His146Tyr/c.436C>T
	58777156		missense_variant	p.Leu235Val/c.703T>G
	58777193		missense_variant	p.Ile247Asn/c.740T>A
	58777501		missense_variant	p.Thr327Met/c.980C>T
	58777661		missense_variant	p.Asp380Glu/c.1140T>A
	58777734		missense_variant	p.Ser405Pro/c.1213T>C
Solyc04g071820.2	58831136	CYP75B1/flavonoid 3-monooxygenase	missense_variant	p.Leu8Ile/c.22C>A
	58831347		missense_variant	p.Ala78Val/c.233C>T
	58832591		missense_variant	p.Ala467Gly/c.1400C>G
	58832666		missense_variant	p.Ser492Tyr/c.1475C>A
Solyc12g045020.1	37475226	CYP84A1/ferulate 5-hydroxylase	missense_variant	p.Arg26Lys/c.77G>A
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Glycoside Hydrolases				
Solyc03g115200.2	65000994	Glucan endo-1 3-beta-glucosidase 1	splice_region_variant	
Solyc04g058080.2	55197152	Carbohydrate-binding X8 domain	missense_variant	p.Ile169Met/c.507A>G
	55197172		missense_variant	p.Val163Met/c.487G>A
	55197226		missense_variant	p.Val145Ile/c.433G>A
	55197226		splice_region_variant	c.433G>A
Solyc07g049370.2	59644207	Glucan endo-1 3-beta-glucosidase A6	missense_variant	p.Ala162Thr/c.484G>A
	59644518		missense_variant	p.Glu265Asp/c.795A>T
Solyc12g008580.1	1971782	Glucan endo-1 3-beta-glucosidase 4	frameshift_variant	p.Leu181_Lys182fs/c.541_542insT
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Hormones				
Solyc01g006540.2	1116398	Lipoxygenase	missense_variant	p.Phe137Ile/c.409T>A
	1118850		splice_region_variant	c.1159G>T
Solyc03g031460.1	4002844	Pentatricopeptide repeat-containing protein	frameshift_variant	p.Lys36fs/c.108_109delAA
Solyc04g080660.2	64771699	JA-o-methyltransferase	missense_variant	p.Ile24Phe/c.70A>T
Solyc09g083110.1	68794138	Pentatricopeptide repeat-containing protein	frameshift_variant	p.Ser641_Lys642fs/c.1923_1924insA
Solyc11g005140.1	129204	Pentatricopeptide repeat-containing protein	missense_variant	p.Glu84Asp/c.252A>T
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Lipid and CHO metabolism				
Solyc04g015270.2	5475953	Glycosyltransferase	missense_variant	p.Lys197Glu/c.589A>G
Solyc04g064490.2	55635636	Glycosyltransferase	missense_variant	p.Gly161Ala/c.482G>C
Solyc07g043500.1	57339024	UDP-glucosyltransferase	stop_lost	p.Ter442Serext*?/c.1325G>C
Solyc09g008060.2	1520830	UDP-glucosyltransferase	frameshift_variant	p.Ile61fs/c.182_183delTA
Solyc10g079320.1	60869271	Glucosyltransferase	missense_variant	p.Thr480Lys/c.1439C>A
	60869686		stop_gained	p.Gln342*/c.1024C>T

Solyc12g009940.1	3101105	UDP-glucosyltransferase	missense_variant	p.Met242Thr/c.725T>C
	3101106		missense_variant	p.Met242Leu/c.724A>T
Solyc12g057060.1	63125665	UDP-glucuronosyltransferase	stop_gained	p.Leu397*/c.1190T>G
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Secondary Metabolism				
Solyc01g088310.2	83083877	Geranylgeranyl reductase	missense_variant	p.Ser102Thr/c.305G>C
	83084607		missense_variant	p.Asp345Glu/c.1035T>G
Solyc02g093230.2	54162564	Caffeoyl-CoA 3-O-methyltransferase	missense_variant	p.Val176Ile/c.526G>A
	54162609		missense_variant	p.Asp161Asn/c.481G>A
	54163239		missense_variant	p.Val32Ala/c.95T>C
	54163423		missense_variant	p.Met2Thr/c.5T>C
Solyc02g093250.2	54173850	Caffeoyl-CoA 3-O-methyltransferase	missense_variant	p.Val32Ala/c.95T>C
	54173850		missense_variant	p.Val32Ala/c.95T>C
Solyc03g025560.2	2960308	Undecaprenyl pyrophosphate synthase (Terpenoid metabolism)	splice_region_variant	
Solyc04g063210.2	55350294	Caffeoyl-CoA 3-O-methyltransferase	missense_variant	p.Asn264Ile/c.791A>T
	55350509		missense_variant	p.Ser192Arg/c.576T>G
Solyc05g017760.2	18312607	Acetyl-CoA C-acetyltransferase/thiolase	missense_variant	p.Gln15Arg/c.44A>G
	18312607		splice_region_variant	c.44A>G
Solyc07g042630.2	56101888	Beta-amyrin synthase/lupeol synthase	missense_variant	p.Glu592Lys/c.1774G>A
	56101900		missense_variant	p.Leu588Met/c.1762T>A
	56102373		missense_variant	p.His532Arg/c.1595A>G
	56102665		missense_variant	p.Leu503Phe/c.1507C>T
	56103772		splice_region_variant	c.1104T>C
	56106210		missense_variant	p.Leu307Pro/c.920T>C
	56106822		missense_variant	p.Trp278Cys/c.834G>C
Solyc07g049660.2	60025633	Hexenol acetyltransferase	missense_variant	p.Lys5Arg/c.14A>G
	60025636		missense_variant	p.Pro6Gln/c.17C>A
	60026053		missense_variant	p.Glu145Gly/c.434A>G
	60027160		splice_donor_variant	c.1415C>T
Solyc08g005640.2	505060	Ent-kaurene/terpenoid synthase	missense_variant	p.Cys34Trp/c.102T>G
	505573		missense_variant	p.Asp87Val/c.260A>T
	505622		missense_variant	p.Ile103Met/c.309A>G
	506851		missense_variant	p.Thr235Ala/c.703A>G
	506925		missense_variant	p.Gln259His/c.777A>T
	507990		missense_variant	p.Ile341Val/c.1021A>G
	507991		missense_variant	p.Ile341Thr/c.1022T>C
	508423		splice_region_variant	c.1305T>C
	519560		missense_variant	p.Ala153Pro/c.457G>C
	519611		missense_variant	p.Thr136Ser/c.406A>T
	519715		missense_variant	p.Ile101Thr/c.302T>C
	519725		missense_variant	p.Ile98Val/c.292A>G
Solyc08g005680.2	531228	Dehydrodolichyl diphosphate synthase (Terpenoid metabolism)	missense_variant	p.Met100Thr/c.299T>C
	531267		missense_variant	p.Phe113Tyr/c.338T>A
	557160		missense_variant	p.Ser235Thr/c.703T>A
	557196		missense_variant	p.Thr247Ala/c.739A>G
	557643		splice_region_variant	
Solyc08g005720.2	579214	Ent-kaurene synthase	missense_variant	p.Lys70Arg/c.209A>G

	579216		missense_variant	p.Tyr71His/c.211T>C
	582377		missense_variant	p.Ile248Val/c.742A>G
	582416		missense_variant	p.Leu261Phe/c.781C>T
	582463		missense_variant	p.Glu276Asp/c.828A>T
	582600		missense_variant	p.Glu288Gln/c.862G>C
	582865		missense_variant	p.Ala316Glu/c.947C>A
	582894		missense_variant	p.Ser326Ala/c.976T>G
Solyc10g008120.2	2266285	Eugenol-o-methyltransferase	missense_variant	p.Val202Ala/c.605T>C
Solyc11g011240.1	4289954	Geranylgeranyl pyrophosphate synthase 1	missense_variant	p.Pro204Leu/c.611C>T
Solyc12g006510.1	1018356	Beta-amyrin synthase	missense_variant	p.Gly79Arg/c.235G>A
Solyc12g006530.1	1046904	Beta-amyrin synthase	splice_donor_variant	c.1573T>A
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Proteases				
Solyc01g010710.2	5744828	Serine carboxypeptidase	missense_variant	p.Gly162Ala/c.485T>C
	5745667		missense_variant	p.Phe85Ser/c.254T>C
	5745923		missense_variant	p.Arg56Ser/c.168A>C
Solyc01g079950.2	79099202	Aspartyl protease family protein	stop_retained_variant	p.Ter399Ter/c.1195C>T
Solyc01g087970.2	82815491	Serine carboxypeptidase	missense_variant	p.Lys257Thr/c.770T>C
Solyc04g015340.2	5529704	Serine carboxypeptidase	missense_variant	p.Gln262His/c.786C>T
	5530212		missense_variant	p.Tyr161His/c.481G>C
Solyc07g005960.2	793484	Serine carboxypeptidase	missense_variant	p.Asn458Tyr/c.1372C>T
	793492		missense_variant	p.Phe455Ser/c.1364G>C
	794421		stop_gained	p.Tyr355*/c.1065T>G
	794797		missense_variant	p.Ala327Thr/c.979G>A
	795244		stop_lost	p.Ter286Tyrext*?/c.858T>C
	797712		missense_variant	p.Leu116Ile/c.346T>A
	799292		missense_variant	p.Ile29Val/c.85C>G
	799333		missense_variant	p.Asp15Val/c.44C>T
	64023963		missense_variant	p.Pro527Ser/c.1579C>T
	64024084		missense_variant	p.Pro517Thr/c.1549C>A
	64024852		missense_variant	p.Val368Ala/c.1103T>C
	64025084		missense_variant	p.Arg291Cys/c.871C>T
	64025336		missense_variant	p.Thr207Ser/c.619A>T
	64027402		missense_variant	p.Gly152Val/c.455G>T
	64027891		missense_variant	p.Asn75Lys/c.225T>G
	64028256		missense_variant	p.Ser24Ile/c.71G>T
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Transcription Factors				
Solyc01g057080.1	58294730	AP2 domain-containing transcription factor, putative	stop_lost	p.Ter114Glnext*?/c.340T>C
	58294940		missense_variant	p.Val44Ile/c.130G>A

Table S4. Genes differentially expressed involved in the response to *Tuta absoluta* affected by deleterious variants in Susceptible genotype.

GENE	POSITION	ANNOTATION	VARIANT TYPE	AA CHANGE
Cytochrome p450				
Solyc02g089160.2	51046486	CYP85A1/BRASSINOSTEROID-6-OXIDASE 1	intron_variant	c.1176-27A>C
	51046733		splice_acceptor_variant	c.1300A>T
	51046733		intron_variant	c.1300-2A>T
Solyc03g122350.2	70222333	CYP75B1/flavonoid 3-monooxygenase	upstream_gene_variant	
	70222975		upstream_gene_variant	
	70223051		upstream_gene_variant	
	70228028		synonymous_variant	p.Gly187Gly/c.561A>G
	70228613		intron_variant	c.897+249G>A
	70228641		intron_variant	c.898-256T>C
	70232137		downstream_gene_variant	
	70232544		downstream_gene_variant	
	70232644		downstream_gene_variant	
	70232645		downstream_gene_variant	
	70233346		downstream_gene_variant	
	70233697		downstream_gene_variant	
	70234106		downstream_gene_variant	
Solyc05g011970.2	5175721	CYP734A1/BAS1 (PHYB ACTIVATION TAGGED SUPPRESSOR 1)/Secologanin synthase	upstream_gene_variant	
	5176174		upstream_gene_variant	
	5177854		upstream_gene_variant	
	5177860		upstream_gene_variant	
	5178575		upstream_gene_variant	
	5178610		upstream_gene_variant	
	5178663		upstream_gene_variant	
	5178829		upstream_gene_variant	
	5178950		upstream_gene_variant	
	5178951		upstream_gene_variant	
	5179165		upstream_gene_variant	
	5179436		upstream_gene_variant	
	5180319		stop_gained	p.Tyr95*/c.285C>A
	5180319		splice_region_variant	c.285C>A
	5180492		intron_variant	c.286+172T>C
	5180595		intron_variant	c.287-102C>T
	5180651		intron_variant	c.287-46G>C
	5180811		missense_variant	p.Lys134Met/c.401A>T
	5181267		intron_variant	c.741+35_741+36insG
	5181331		stop_lost	p.Ter254Argext*?/c.760T>C
	5181636		synonymous_variant	p.Phe355Phe/c.1065T>C
	5182121		synonymous_variant	p.Lys471Lys/c.1413A>G
	5182353		downstream_gene_variant	
Solyc11g007980.1	2187052	CYP71B35/monooxygenase	upstream_gene_variant	
	2187359		upstream_gene_variant	
	2188219		upstream_gene_variant	
	2188699		upstream_gene_variant	

	2192116		missense_variant	p.Ile90Val/c.268A>G
	2192121		missense_variant	p.Phe91Leu/c.273C>A
	2192959		missense_variant	p.Asp198Ala/c.593A>C
	2193403		missense_variant	p.Ala309Thr/c.925G>A
	2193428		missense_variant	p.Ile317Thr/c.950T>C
	2193660		synonymous_variant	p.Ile394Ile/c.1182C>T
	2198478		downstream_gene_variant	
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
HORMONES METABOLISM				
Solyc01g006560.2	1124262	Lipoxygenase (downregulated DEG)	downstream_gene_variant	
	1124351		downstream_gene_variant	
	1124507		downstream_gene_variant	
	1124571		downstream_gene_variant	
	1124605		downstream_gene_variant	
	1124700		downstream_gene_variant	
	1124701		downstream_gene_variant	
	1125018		downstream_gene_variant	
	1125192		downstream_gene_variant	
	1125606		downstream_gene_variant	
	1125710		downstream_gene_variant	
	1125718		downstream_gene_variant	
	1125782		downstream_gene_variant	
	1125954		downstream_gene_variant	
	1125963		downstream_gene_variant	
	1126312		downstream_gene_variant	
	1126316		downstream_gene_variant	
	1126611		downstream_gene_variant	
	1126624		downstream_gene_variant	
	1126674		downstream_gene_variant	
	1126681		downstream_gene_variant	
	1126762		downstream_gene_variant	
	1126775		downstream_gene_variant	
	1126929		downstream_gene_variant	
	1128932		3_prime_UTR_variant	c.*3_*4insTA
	1129030		synonymous_variant	p.Lys868Lys/c.2604A>G
	1129060		missense_variant	p.Gln858His/c.2574C>T
	1129399		intron_variant	c.2281-46C>T
	1129401		intron_variant	c.2281-48T>C
	1129529		intron_variant	c.2281-176C>A
	1129542		intron_variant	c.2281-189A>G
	1129611		intron_variant	c.2281-258T>G
	1130015		intron_variant	c.2280+144A>C
	1130020		intron_variant	c.2280+139T>C
	1132534		missense_variant	p.Val459Asp/c.1376G>A
	1132534		splice_region_variant	c.1376G>A
	1132720		intron_variant	c.1375-185A>G
	1132846		intron_variant	c.1375-311T>C

	1133199		intron_variant	c.1374+66C>G
	1133340		missense_variant	p.Ile433Met/c.1299A>G
	1133345		missense_variant	p.Gly432Ser/c.1294G>A
	1134171		synonymous_variant	p.Leu298Leu/c.894T>A
	1134325		intron_variant	c.864-124C>T
	1134369		intron_variant	c.864-168T>G
	1134622		intron_variant	c.864-421T>C
	1135095		missense_variant	p.Ile274Phe/c.820G>T
	1135143		missense_variant	p.Val258Leu/c.772T>C
	1135149		missense_variant	p.Val256Leu/c.766C>T
	1135308		intron_variant	c.621-14C>T
	1135309		intron_variant	c.621-15T>A
	1136622		intron_variant	c.620+23C>T
	1136630		intron_variant	c.620+15C>T
	1138475		intron_variant	c.352+649T>C
	1139095		intron_variant	c.352+29T>C
	1139134		synonymous_variant	p.Ala114Ala/c.342G>T
	1139376		synonymous_variant	p.Phe34Phe/c.100A>T
	1139475		5_prime_UTR_variant	c.-2_-3insAT
Solyc01g087560.2	82508213	S-adenosyl-methionine-sterol-C- methyltransferase (UP)	intron_variant	c.84-558delT
	82508338		intron_variant	c.84-436G>A
	82508376		intron_variant	c.84-398G>A
	82508493		intron_variant	c.84-281T>C
	82510020		synonymous_variant	p.Arg76Arg/c.228A>G
	82510051		stop_lost	p.Ter87Argext*?/c.259T>C
	82510057		missense_variant	p.Leu89Phe/c.265C>T
	82510858		intron_variant	c.359-58T>G
	82510860		intron_variant	c.359-56G>A
	82512385		intron_variant	c.868-233T>G
	82512641		synonymous_variant	p.Gly297Gly/c.891T>A
	82512757		intron_variant	c.965+42T>C
	82512775		intron_variant	c.966-38T>G
	82512949		3_prime_UTR_variant	c.*75T>A
	82513221		downstream_gene_variant	
Solyc01g095110.2	86503542	Pentatricopeptide repeat (PPR) superfamily protein	missense_variant	p.Cys669Arg/c.2005T>C
	86503554		missense_variant	p.Val665Ile/c.1993G>A
	86503733		missense_variant	p.Ala605Val/c.1814C>T
	86503734		missense_variant	p.Ala605Ser/c.1813G>T
	86503908		missense_variant	p.Arg547Gly/c.1639A>G
	86504173		synonymous_variant	p.Cys458Cys/c.1374C>T
	86504415		missense_variant	p.Leu378Phe/c.1132C>T
	86504763		missense_variant	p.Val262Met/c.784G>A
	86505004		synonymous_variant	p.Leu181Leu/c.543G>A
Solyc01g099160.2	89487609	Lipoxygenase	downstream_gene_variant	
Solyc01g103160.2	91822525	GLUTAMINE-RICH PROTEIN23	downstream_gene_variant	
	91828946		upstream_gene_variant	
	91830765		upstream_gene_variant	

Solyc01g107860.2	95245846	Cystathionine beta-synthase	downstream_gene_variant	
Solyc01g109140.2	96193066	cytochrome p450/allene oxide synthase	missense_variant	p.Lys135Glu/c.403A>G
	96193104		synonymous_variant	p.Thr147Thr/c.441G>A
	96193993		stop_gained	p.Gly394*/c.1180G>T
	96194079		synonymous_variant	p.Glu422Glu/c.1266G>A
	96194161		stop_gained	p.Gln450*/c.1348C>T
Solyc01g110570.2	97225899	Auxin responsive SAUR protein	intron_variant	c.*93+51_*93+50insTT
	97225899		intron_variant	c.*93+51_*93+50insTT
	97225899		intron_variant	c.*93+51_*93+50insT
	97225947		splice_donor_variant	c.412_413insTT
	97225947		splice_donor_variant	c.412_413insT
	97225947		intron_variant	c.*93+2_*93+1insTT
	97225947		intron_variant	c.*93+2_*93+1insT
	97226026		3_prime_UTR_variant	c.*25T>C
	97226376		5_prime_UTR_variant	c.-8A>G
Solyc01g110580.2	97234737	Auxin responsive SAUR protein	downstream_gene_variant	
	97234738		downstream_gene_variant	
	97234739		downstream_gene_variant	
	97234801		3_prime_UTR_variant	c.*129_*130insT
	97234801		3_prime_UTR_variant	c.*129_*130insTT
Solyc01g110590.2	97240563	Auxin responsive SAUR protein	downstream_gene_variant	
	97240703		downstream_gene_variant	
	97240779		synonymous_variant	p.Asp85Asp/c.255C>T
	97240833		synonymous_variant	p.Pro67Pro/c.201T>C
	97240870		missense_variant	p.Ser55Thr/c.164G>C
	97240890		synonymous_variant	p.Val48Val/c.144T>A
	97240899		synonymous_variant	p.His45His/c.135C>T
	97240899		synonymous_variant	p.His45His/c.135C>T
	97240928		missense_variant	p.Arg36Cys/c.106C>T
Solyc01g110660.2	97294678	Auxin responsive SAUR protein	synonymous_variant	p.Pro34Pro/c.102A>G
	97294687		synonymous_variant	p.Phe31Phe/c.93T>C
	97294753		synonymous_variant	p.Pro9Pro/c.27G>A
	97294832		5_prime_UTR_variant	c.-53C>A
	97294835		5_prime_UTR_variant	c.-56G>A
	97296264		upstream_gene_variant	
	97296661		upstream_gene_variant	
	97296662		upstream_gene_variant	
Solyc01g110940.2	97400083	Auxin responsive SAUR protein	upstream_gene_variant	
	97400324		upstream_gene_variant	
	97400452		upstream_gene_variant	
	97400582		upstream_gene_variant	
	97402412		upstream_gene_variant	
	97402423		upstream_gene_variant	
	97402459		upstream_gene_variant	
	97402467		upstream_gene_variant	
	97402681		upstream_gene_variant	
	97403222		upstream_gene_variant	

	97404239		5_prime_UTR_variant	c.-155G>A
	97404412		missense_variant	p.Arg7Ser/c.19C>A
	97404412		missense_variant	p.Arg7Ser/c.19C>A
	97404730		3_prime_UTR_variant	c.*79T>C
	97404895		downstream_gene_variant	
	97408725		downstream_gene_variant	
	97409339		downstream_gene_variant	
	97409587		downstream_gene_variant	
	97409710		downstream_gene_variant	
	97409723		downstream_gene_variant	
Solyc02g069490.2	39357781	FAD linked oxidase domain protein	missense_variant	p.Leu565Ser/c.1694T>C
	39357781		splice_region_variant	c.1694T>C
	39357782		missense_variant	p.Leu565Val/c.1693T>G
	39357782		splice_region_variant	c.1693T>G
	39358343		intron_variant	c.1257+44G>T
Solyc03g031460.1	4004715	PPR repeat containing	downstream_gene_variant	
Solyc03g083280.2	53120040	PPR repeat containing	downstream_gene_variant	
Solyc03g114920.1	64784966	PPR repeat containing	upstream_gene_variant	
Solyc03g117570.2	66698088	pentatricopeptide (PPR) repeat-containing protein	synonymous_variant	p.Leu152Leu/c.456T>C
	66700051		upstream_gene_variant	
	66700495		upstream_gene_variant	
	66701866		upstream_gene_variant	
Solyc08g069010.2	58086355	pentatricopeptide (PPR) repeat-containing protein	upstream_gene_variant	
	58086355		upstream_gene_variant	
	58106378		downstream_gene_variant	
	58107047		downstream_gene_variant	
	58107138		downstream_gene_variant	
Solyc09g055900.2	46064706	Lipoxygenase (downregulated DEG)	upstream_gene_variant	
	46074510		downstream_gene_variant	
	46074816		downstream_gene_variant	
Solyc10g005280.1	208688	PPR336 (pentatricopeptide repeat 336)	upstream_gene_variant	
	208707		upstream_gene_variant	
	210379		upstream_gene_variant	
	214779		downstream_gene_variant	
	215535		downstream_gene_variant	
	215701		downstream_gene_variant	
	215922		downstream_gene_variant	
	216526		downstream_gene_variant	
	216798		downstream_gene_variant	
	216973		downstream_gene_variant	
	217112		downstream_gene_variant	
	217145		downstream_gene_variant	
	217485		downstream_gene_variant	
	218064		downstream_gene_variant	
	218785		downstream_gene_variant	
Solyc10g086220.1	65116245	12-oxophytodienoate reductase (downregulated DEG)	downstream_gene_variant	
	65116305		downstream_gene_variant	

	65116390		downstream_gene_variant	
	65117078		downstream_gene_variant	
	65117113		downstream_gene_variant	
	65117696		downstream_gene_variant	
	65119516		downstream_gene_variant	
	65119638		downstream_gene_variant	
	65119800		downstream_gene_variant	
	65119923		downstream_gene_variant	
	65120527		downstream_gene_variant	
	65120843		downstream_gene_variant	
	65121049		missense_variant	p.Glu313Gly/c.938A>G
	65121066		synonymous_variant	p.Ala307Ala/c.921T>C
	65121096		synonymous_variant	p.Arg297Arg/c.891G>A
	65121664		intron_variant	c.362-40delA
	65121810		intron_variant	c.361+18G>A
	65121813		intron_variant	c.361+15A>C
	65121999		missense_variant	p.Leu64Val/c.190C>G
	65122130		missense_variant	p.Cys20Tyr/c.59G>A
	65122151		missense_variant	p.Lys13Met/c.38A>T
	65124391		upstream_gene_variant	
	65124685		upstream_gene_variant	
	65124698		upstream_gene_variant	
	65125408		upstream_gene_variant	
	65125758		upstream_gene_variant	
	65125911		upstream_gene_variant	
	65126274		upstream_gene_variant	
	65126297		upstream_gene_variant	
Solyc11g005140.1	128937	pentatricopeptide (PPR) repeat-containing protein	upstream_gene_variant	
Solyc12g009220.1	2504629	Jasmonate ZIM-domain protein 1 OS-Solanum lycopersicum PE-2 SV-1	downstream_gene_variant	
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Glycoside Hydrolases				
Solyc10g079860.1	61325309	Glucan endo-1 3-beta-glucosidase (up)	downstream_gene_variant	
	61325520		downstream_gene_variant	
	61325548		downstream_gene_variant	
	61325632		downstream_gene_variant	
	61326506		downstream_gene_variant	
	61326765		downstream_gene_variant	
	61326840		downstream_gene_variant	
	61326885		downstream_gene_variant	
	61327117		downstream_gene_variant	
	61327688		downstream_gene_variant	
	61328384		downstream_gene_variant	
	61328477		downstream_gene_variant	
	61329164		downstream_gene_variant	
	61329653		downstream_gene_variant	
	61329656		downstream_gene_variant	
	61329788		downstream_gene_variant	

	61329930		downstream_gene_variant	
	61330271		synonymous_variant	p.Ser260Ser/c.780T>C
	61330835		synonymous_variant	p.Ala72Ala/c.216C>T
	61331005		intron_variant	c.83-37T>C
Solyc11g068440.1	53174710	Glucan endo-1%2C3-beta-glucosidase 11 OS-Arabidopsis thaliana GN-At1g32860 PE-1 SV-1 (up)	downstream_gene_variant	
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
SECONDARY METABOLISM				
Solyc01g105920.2	93956704	Ocimene synthase, putative	stop_gained	p.Glu260*/c.778G>T
Solyc03g042560.1	6965017	PAL 2 (upregulated)	missense_variant	p.Leu29Phe/c.85C>T
	6965053		missense_variant	p.Ala41Thr/c.121G>A
	6965212		missense_variant	p.Lys94Gln/c.280A>C
	6965224		missense_variant	p.Lys98Gln/c.292A>C
	6965254		stop_gained	p.Gln108*/c.322C>T
	6965345		missense_variant	p.Gly138Asp/c.413G>A
	6965358		synonymous_variant	p.Asn142Asn/c.426C>T
	6965419		stop_gained	p.Arg163*/c.487A>T
	6965465		missense_variant	p.Leu178Ser/c.533T>C
	6965516		missense_variant	p.Val195Ala/c.584T>C
	6965523		missense_variant	p.Ser197Arg/c.591T>G
	6965623		stop_gained	p.Gln231*/c.691C>T
	6965689		missense_variant	p.Glu253Lys/c.757G>A
	6965695		missense_variant	p.Val255Ile/c.763G>A
	6965918		missense_variant	p.Val329Ala/c.986T>C
	6965928		synonymous_variant	p.Pro332Pro/c.996T>C
	6966103		missense_variant	p.Val391Phe/c.1171G>T
	6966225		synonymous_variant	p.Pro431Pro/c.1293C>T
	6966258		missense_variant	p.Met442Ile/c.1326G>A
	6966303		missense_variant	p.Arg457Ser/c.1371A>C
	6966336		downstream_gene_variant	
Solyc03g097030.2	59385903	4-coumarate CoA ligase (downregulated)	missense_variant	p.Asp208Gly/c.623A>G
Solyc03g097170.2	59489648	Cinnamoyl-CoA reductase-related (phenylethanol pathway-UP)	upstream_gene_variant	
	59489769		upstream_gene_variant	
	59490470		upstream_gene_variant	
	59495403		intron_variant	c.155-76G>T
	59501169		downstream_gene_variant	
Solyc11g069050.1	53626544	4-coumarate CoA ligase (downregulated)	upstream_gene_variant	
	53626544		upstream_gene_variant	
	53627755		intron_variant	c.996+188C>T
	53629809		intron_variant	c.1187-1023G>T
	53631332		intron_variant	c.1332+355C>T
	53633350		downstream_gene_variant	
Solyc12g006530.1	1040934	beta-amyrin synthase, putative (downregulated DEG)	upstream_gene_variant	
	1041120		synonymous_variant	p.Arg24Arg/c.70A>C
	1041188		synonymous_variant	p.Arg46Arg/c.138A>T
	1041189		missense_variant	p.Lys47Gln/c.139A>C
	1042561		intron_variant	c.202-1228A>G
	1044004		intron_variant	c.387+30C>T

	1046246		missense_variant	p.Ser397Pro/c.1189T>C
	1046364		intron_variant	c.1231-20A>T
	1046970		intron_variant	c.1573-58T>A
	1047796		intron_variant	c.1998+85T>C
	1048033		intron_variant	c.1999-128A>T
	1048056		intron_variant	c.1999-105T>C
	1048470		intron_variant	c.2163+145A>T
	1048479		intron_variant	c.2163+154T>C
	1048556		intron_variant	c.2163+231C>T
	1048589		intron_variant	c.2163+264T>A
	1048626		intron_variant	c.2164-250delA
	1048743		intron_variant	c.2164-136G>A
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Lipid and CHO metabolism				
Solyc01g095960.2	87072542	O-acyltransferase (up-regulated DEG)	upstream_gene_variant	
	87076018		missense_variant	p.Phe175Ser/c.524T>C
	87076460		missense_variant	p.Leu289Phe/c.865C>T
	87076986		missense_variant	p.Val353Met/c.1057G>A
	87077928		downstream_gene_variant	
	87082049		downstream_gene_variant	
	87082104		downstream_gene_variant	
	87082197		downstream_gene_variant	
	87082239		downstream_gene_variant	
	87082281		downstream_gene_variant	
Solyc01g107780.2	95200178	UDP-glucuronosyl/UDP-glucosyltransferase	downstream_gene_variant	
	95200682		downstream_gene_variant	
	95202150		downstream_gene_variant	
	95202268		downstream_gene_variant	
	95202329		downstream_gene_variant	
	95202359		downstream_gene_variant	
	95203920		synonymous_variant	p.Leu473Leu/c.1419A>G
	95204572		missense_variant	p.Glu256Val/c.767A>T
Solyc03g078730.1	51426713	UDP-glucuronosyl/UDP-glucosyltransferase (down-regulated)	upstream_gene_variant	
Solyc04g077470.2	62386209	Glycosyl transferase family 2 (up-reg)	synonymous_variant	p.Val407Val/c.1221T>G
	62386209		synonymous_variant	p.Val407Val/c.1221T>G
	62386873		synonymous_variant	p.Leu561Leu/c.1683T>C
	62387185		synonymous_variant	p.Ser665Ser/c.1995C>A
	62387733		downstream_gene_variant	
	62387828		downstream_gene_variant	
	62387920		downstream_gene_variant	
	62388005		downstream_gene_variant	
	62388174		downstream_gene_variant	
Solyc05g009820.2	4035842	Glycosyl transferase family 8 (upregulated)	missense_variant	p.Thr21Pro/c.61A>C
	4035842		missense_variant	p.Thr21Pro/c.61A>C
	4036569		intron_variant	c.773+15G>A
	4036667		intron_variant	c.773+113T>C
	4037121		3_prime_UTR_variant	c.*51G>A

	4037220		3_prime_UTR_variant	c.*150C>T
	4037350		downstream_gene_variant	
	4037399		downstream_gene_variant	
	4037449		downstream_gene_variant	
	4037573		downstream_gene_variant	
Solyc08g078650.2	62410393	Glycosyl transferase family 8 (up)	synonymous_variant	p.Tyr128Tyr/c.383T>A
Solyc09g098080.2	71986651	UDP-glucuronosyl/UDP-glucosyltransferase	intron_variant	c.900-31_900-30delAA
	71990723		downstream_gene_variant	
Solyc10g085230.1	64495464	UDP-glucuronosyltransferase (downregulated deg)	upstream_gene_variant	
	64495991		upstream_gene_variant	
	64496945		upstream_gene_variant	
	64500218		missense_variant	p.Ile29Val/c.85A>G
	64500418		missense_variant	p.Met95Ile/c.285G>A
	64500419		missense_variant	p.Met96Leu/c.286A>T
	64500651		intron_variant	c.463+56_463+57insC
	64501301		synonymous_variant	p.Gly342Gly/c.1026G>C
	64501364		synonymous_variant	p.Val363Val/c.1089G>A
	64501453		missense_variant	p.Arg393Lys/c.1178G>A
	64501566		missense_variant	p.Cys431Gly/c.1291T>G
	64504282		downstream_gene_variant	
	64505679		downstream_gene_variant	
Solyc11g012260.1	5127749	Acyltransferase (down-reg)	downstream_gene_variant	
	5130868		downstream_gene_variant	
	5132175		downstream_gene_variant	
	5132506		missense_variant	p.Ile380Met/c.1140C>G
	5133547		synonymous_variant	p.Tyr33Tyr/c.99T>C
	5133682		upstream_gene_variant	
	5135754		upstream_gene_variant	
	5136228		upstream_gene_variant	
	5137015		upstream_gene_variant	
Solyc11g072990.1	56124250	Acyltransferase	downstream_gene_variant	
	56124256		downstream_gene_variant	
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Transcription factors				
Solyc09g065100.1	62956299	SlbHLH150	missense_variant	p.Ala636Ser/c.1906G>T

Table S5. Genes differentially expressed involved in the response to *Tuta absoluta* affected by deleterious variants in F1 hybrid genotype.

GENE	POSITION	ANNOTATION	VARIANT TYPE	AA CHANGE
Hormones Metabolism				
Solyc01g110570.2	97225899	Auxin responsive SAUR protein	intron_variant	c.*93+51_*93+50insTT
	97225947		splice_donor_variant	c.412_413insTT
	97225947		splice_donor_variant	c.412_413insT
	97225947		intron_variant	c.*93+2_*93+1insTT
	97225947		intron_variant	c.*93+2_*93+1insT
	97226026		3_prime_UTR_variant	c.*25T>C
	97226376		5_prime_UTR_variant	c.-8A>G
Solyc01g110580.2	97234739	Auxin responsive SAUR protein	downstream_gene_variant	
Solyc01g110590.2	97240563	Auxin responsive SAUR protein	downstream_gene_variant	
Solyc02g063240.2	35298495	Fatty acid hydrolase	upstream_gene_variant	
Solyc03g031460.1	4004715	Pentatricopeptide repeat-containing protein	downstream_gene_variant	
Solyc04g055260.2	53898446	SAM dependent carboxyl methyltransferase	missense_variant	p.Glu328Lys/c.982G>A
	53899855		splice_region_variant	c.759A>G
	53899855		synonymous_variant	p.Gln253Gln/c.759A>G
	53900531		frameshift_variant	p.Leu154fs/c.462delA
	53900629		missense_variant	p.Asn122Ser/c.365A>G
Solyc06g008810.2	2751123	Leucine-rich repeat cysteine-containing subtype	3_prime_UTR_variant	c.*199A>G
	2751331		frameshift_variant	p.Phe417fs/c.1250delT
	2751530		missense_variant	p.Ile368Met/c.1104T>G
	2751530		splice_region_variant	c.1104T>G
	2751705		missense_variant	p.Gly310Glu/c.929G>A
	2751733		synonymous_variant	p.Leu301Leu/c.901C>T
	2754877		5_prime_UTR_premature_start_codon_gain_variant	
	2754877		5_prime_UTR_variant	c.-778C>T
Solyc07g056570.1	64361087	9-cis-epoxycarotenoid dioxygenase (downregulated)	downstream_gene_variant	
	64361207		downstream_gene_variant	
	64361418		synonymous_variant	p.Lys582Lys/c.1746G>A
	64361433		synonymous_variant	p.Leu577Leu/c.1731G>A
	64361691		synonymous_variant	p.Leu491Leu/c.1473C>T
	64361894		missense_variant	p.Ala424Pro/c.1270G>C
	64361988		synonymous_variant	p.Gly392Gly/c.1176T>G
Solyc08g016720.1	8734740	9-cis-epoxycarotenoid dioxygenase (downregulated)	missense_variant	p.His150Tyr/c.448C>T
Solyc09g083110.1	68791138	Pentatricopeptide (PPR) repeat-containing protein	downstream_gene_variant	
	68791314		downstream_gene_variant	
	68791335		downstream_gene_variant	
	68792301		downstream_gene_variant	
	68792908		downstream_gene_variant	
	68795137		missense_variant	p.Gln311Glu/c.931C>G
	68796627		upstream_gene_variant	
	68797804		upstream_gene_variant	
	68799383		upstream_gene_variant	
	68800041		upstream_gene_variant	

Solyc11g005140.1	128937	Pentatricopeptide (PPR) repeat-containing protein	upstream_gene_variant	
	129204		missense_variant	p.Glu84Asp/c.252A>T
Solyc12g011040.1	3892608	Lipoxygenase (up)	downstream_gene_variant	
	3892611		downstream_gene_variant	
	3892758		downstream_gene_variant	
	3892770		downstream_gene_variant	
	3892855		downstream_gene_variant	
	3892856		downstream_gene_variant	
	3892905		downstream_gene_variant	
	3892944		downstream_gene_variant	
	3893293		downstream_gene_variant	
	3893529		downstream_gene_variant	
	3893530		downstream_gene_variant	
	3893560		downstream_gene_variant	
	3893582		downstream_gene_variant	
	3893678		downstream_gene_variant	
	3894263		synonymous_variant	p.Pro830Pro/c.2490T>C
	3894286		missense_variant	p.Glu823Gln/c.2467G>C
	3894507		intron_variant	c.2261-15A>C
	3896565		synonymous_variant	p.Ile562Ile/c.1686T>A
	3899653		synonymous_variant	p.Asn350Asn/c.1050T>C
	3899695		synonymous_variant	p.Asp336Asp/c.1008T>C
	3899768		missense_variant	p.Gln312Arg/c.935A>G
	3903974		missense_variant	p.Leu103Val/c.307T>G
GENE	**POSITION**	**ANNOTATION**	**VARIANT TYPE**	**AA CHANGE**
Secondary Metabolism				
Solyc03g025560.2	2960401	Undecaprenyl pyrophosphate synthetase family protein	intron_variant	c.-43+94delG
	2961249		synonymous_variant	p.Ser100Ser/c.300A>G
	2962664		downstream_gene_variant	
	2962919		downstream_gene_variant	
	2964615		downstream_gene_variant	
	2966439		downstream_gene_variant	
	2966456		downstream_gene_variant	
Solyc04g063210.2	55345930	Caffeoyl-CoA 3-O-methyltransferase	downstream_gene_variant	
	55345983		downstream_gene_variant	
	55346046		downstream_gene_variant	
	55347934		downstream_gene_variant	
	55350097		3_prime_UTR_variant	c.*139G>A
	55350146		3_prime_UTR_variant	c.*90A>G
	55350169		3_prime_UTR_variant	c.*67A>G
	55350294		missense_variant	p.Asn264Ile/c.791A>T
	55350509		missense_variant	p.Ser192Arg/c.576T>G
	55350848		synonymous_variant	p.Pro79Pro/c.237C>T
	55350908		intron_variant	c.202-25A>G
	55351228		intron_variant	c.144+125C>T
	55351515		5_prime_UTR_variant	c.-19A>G

	55351588		intron_variant	c.-29-71_-29-72insCT
	55351591		intron_variant	c.-29-67_-29-68insCC
	55351767		intron_variant	c.-30+127G>A
	55351782		intron_variant	c.-30+112C>T
Solyc05g017760.2	18311208	Acetyl-CoA C-acetyltransferase/thiolase	upstream_gene_variant	
	18312607		missense_variant	p.Gln15Arg/c.44A>G
	18312607		splice_region_variant	c.44A>G
	18316468		intron_variant	c.*20+228T>G
	18316469		intron_variant	c.*20+229G>T
	18316938		3_prime_UTR_variant	c.*35T>C
	18319551		downstream_gene_variant	
Solyc08g005640.2	505573	Ent-kaurene/terpenoid synthase	missense_variant	p.Asp87Val/c.260A>T
	505622		missense_variant	p.Ile103Met/c.309A>G
	506851		missense_variant	p.Thr235Ala/c.703A>G
	506925		missense_variant	p.Gln259His/c.777A>T
	506949		synonymous_variant	p.Lys267Lys/c.801G>A
	506970		synonymous_variant	p.Thr274Thr/c.822A>T
	511686		downstream_gene_variant	
	512341		downstream_gene_variant	
Solyc10g008120.2	2270851	Eugenol-o-methyltransferase	downstream_gene_variant	
	2271032		downstream_gene_variant	
	2271506		downstream_gene_variant	
	2271714		downstream_gene_variant	
	2271881		downstream_gene_variant	
	2271904		downstream_gene_variant	
	2272116		downstream_gene_variant	
	2272428		downstream_gene_variant	
Solyc11g011240.1	4289050	Geranylgeranyl pyrophosphate synthase 1	upstream_gene_variant	
	4292103		downstream_gene_variant	

GENE	POSITION	ANNOTATION	VARIANT TYPE	AA CHANGE
Protease activity				
Solyc01g010710.2	5742178	Serine carboxypeptidase	intron_variant	c.1234+692T>C
	5742210		intron_variant	c.1234+660T>A
	5742746		intron_variant	c.1234+124A>G
	5742756		intron_variant	c.1234+114G>A
	5744828		missense_variant	p.Gly162Ala/c.485T>C
	5745667		missense_variant	p.Phe85Ser/c.254T>C
	5745923		missense_variant	p.Arg56Ser/c.168A>C
	5745943		synonymous_variant	p.Ser50Ser/c.148G>T
Solyc04g015340.2	5522712	Serine carboxypeptidase	downstream_gene_variant	
	5523390		downstream_gene_variant	
	5525620		downstream_gene_variant	
	5525666		downstream_gene_variant	
	5525678		downstream_gene_variant	
	5525902		downstream_gene_variant	
	5525924		downstream_gene_variant	
	5525937		downstream_gene_variant	

5526473	3_prime_UTR_variant	c.*245C>T
5527275	intron_variant	c.1119-196G>C
5527427	intron_variant	c.1119-348G>A
5527446	intron_variant	c.1119-367C>T
5527682	intron_variant	c.1118+352C>T
5527841	intron_variant	c.1118+177_1118+176insTC
5528599	intron_variant	c.898-259T>C
5528716	intron_variant	c.898-376T>A
5528914	intron_variant	c.898-574A>G
5529022	intron_variant	c.897+571G>C
5529118	intron_variant	c.897+475C>A
5529704	missense_variant	p.Gln262His/c.786C>T
5530212	missense_variant	p.Tyr161His/c.481G>C
5531454	5_prime_UTR_variant	c.-144G>A
5531695	upstream_gene_variant	

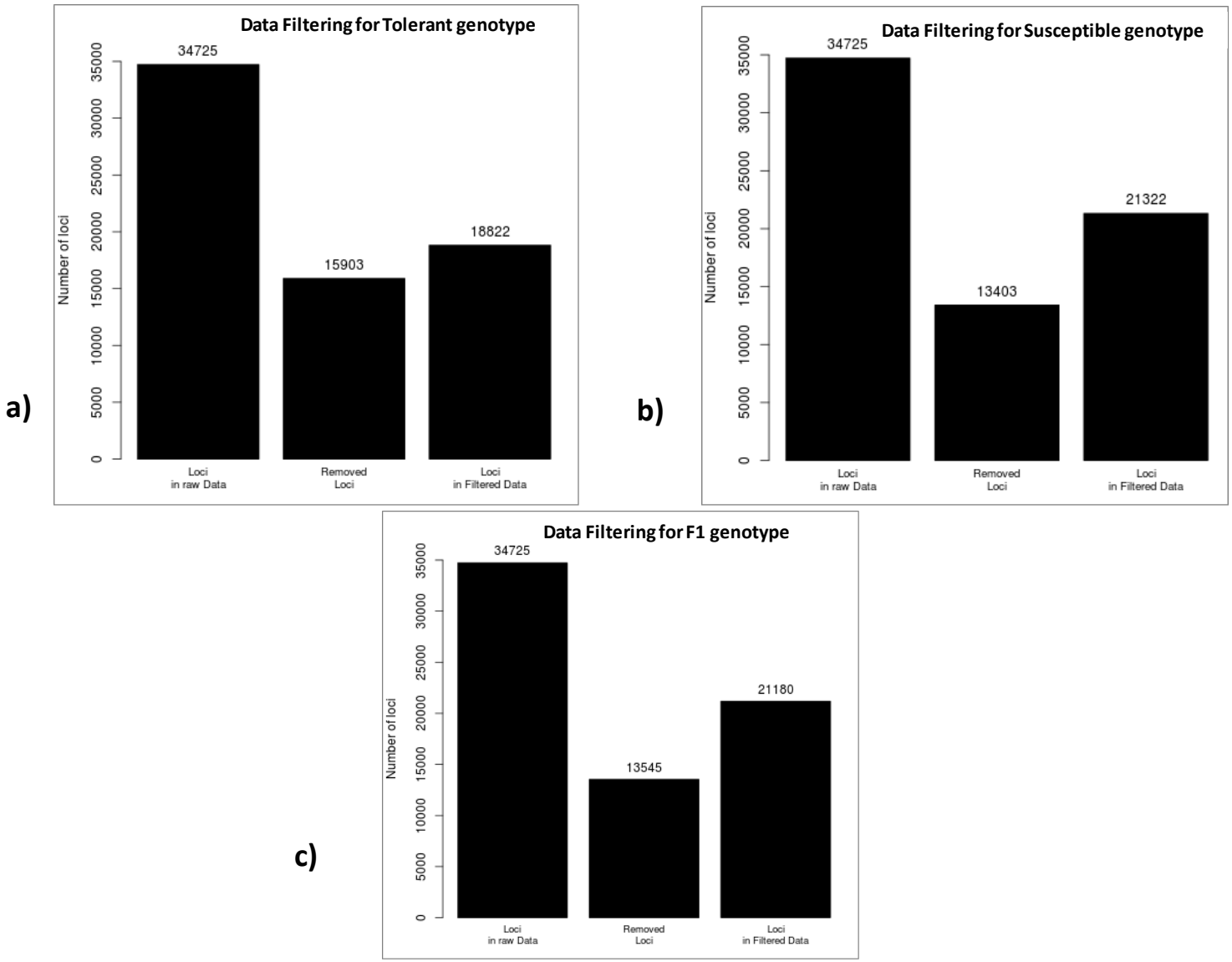
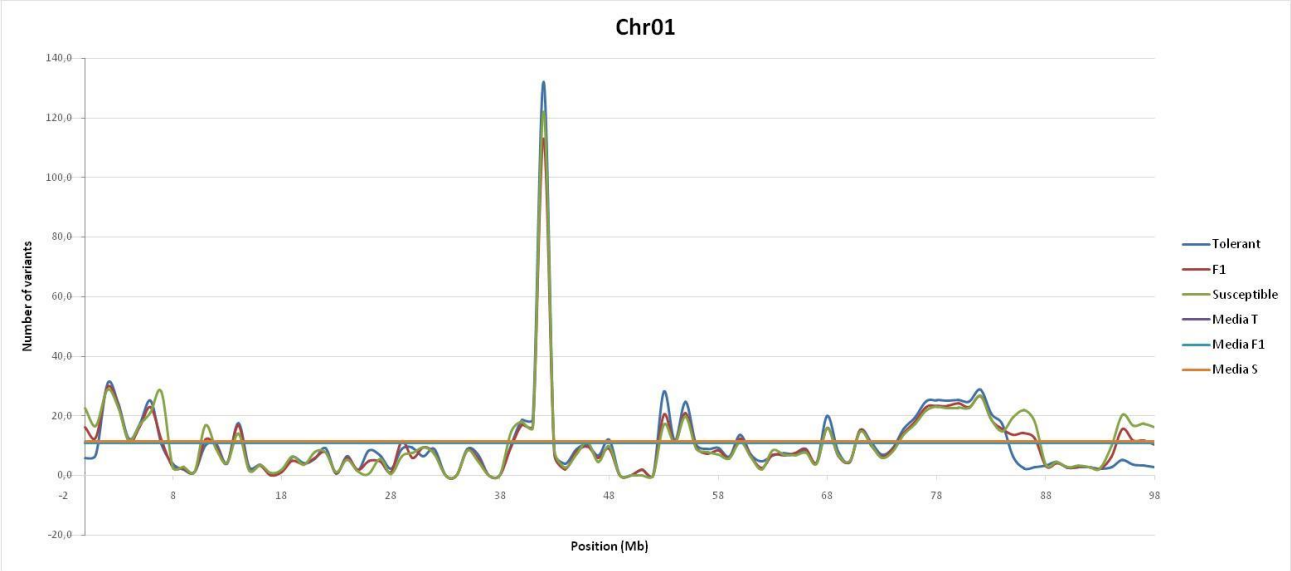


Figure S2. Graph showing the number of loci in the raw data, the removed loci according to HTSFilter analysis and the ones retained for further analysis for the Tolerant (a), Susceptible (b) and F1 (c) genotypes.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80,0
60,0
40,0
20,0
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-20,0
Number of variants
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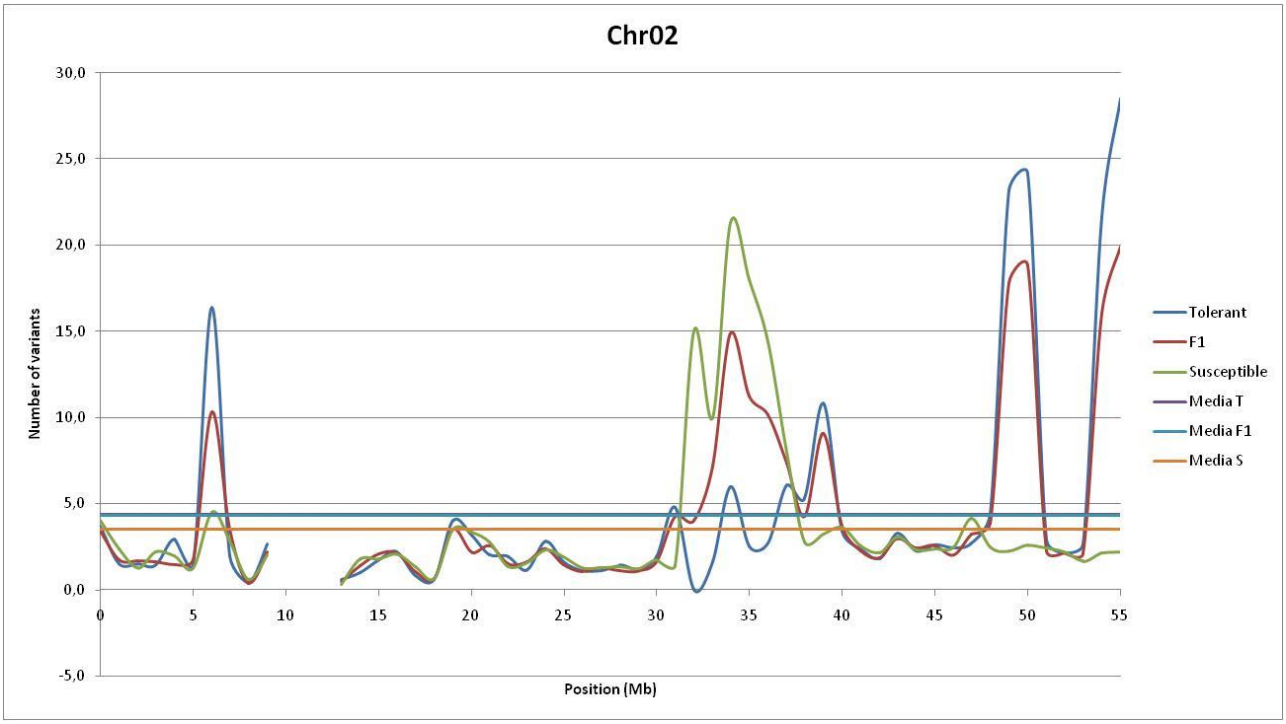
18
28
38
48
58
68
78
88
98
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S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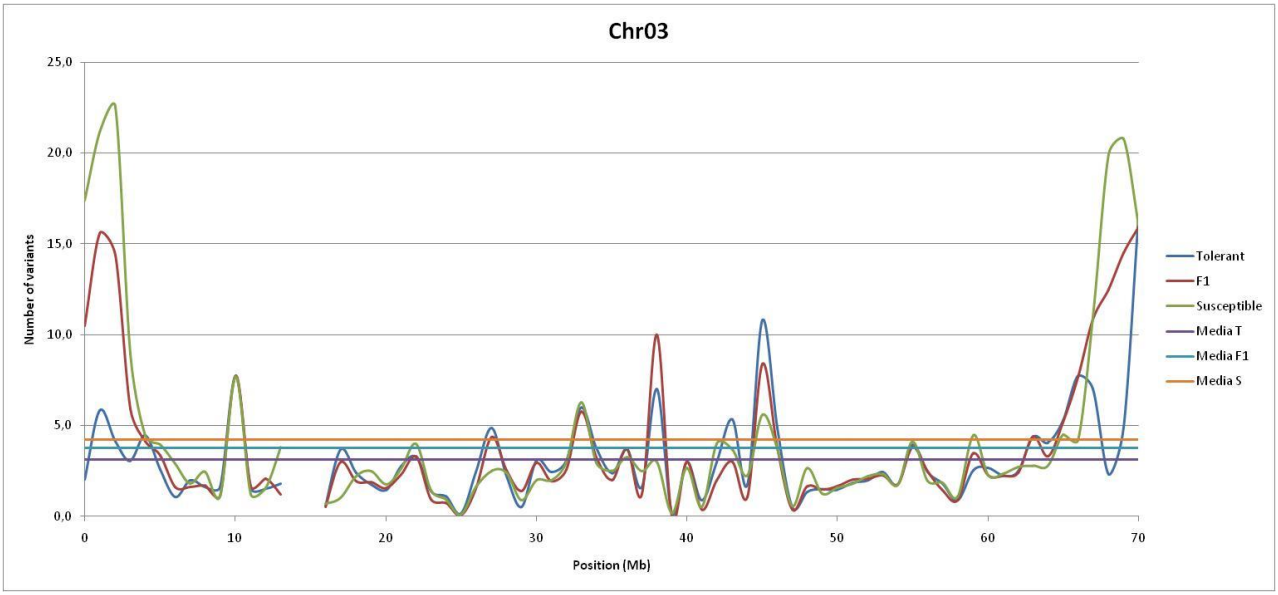
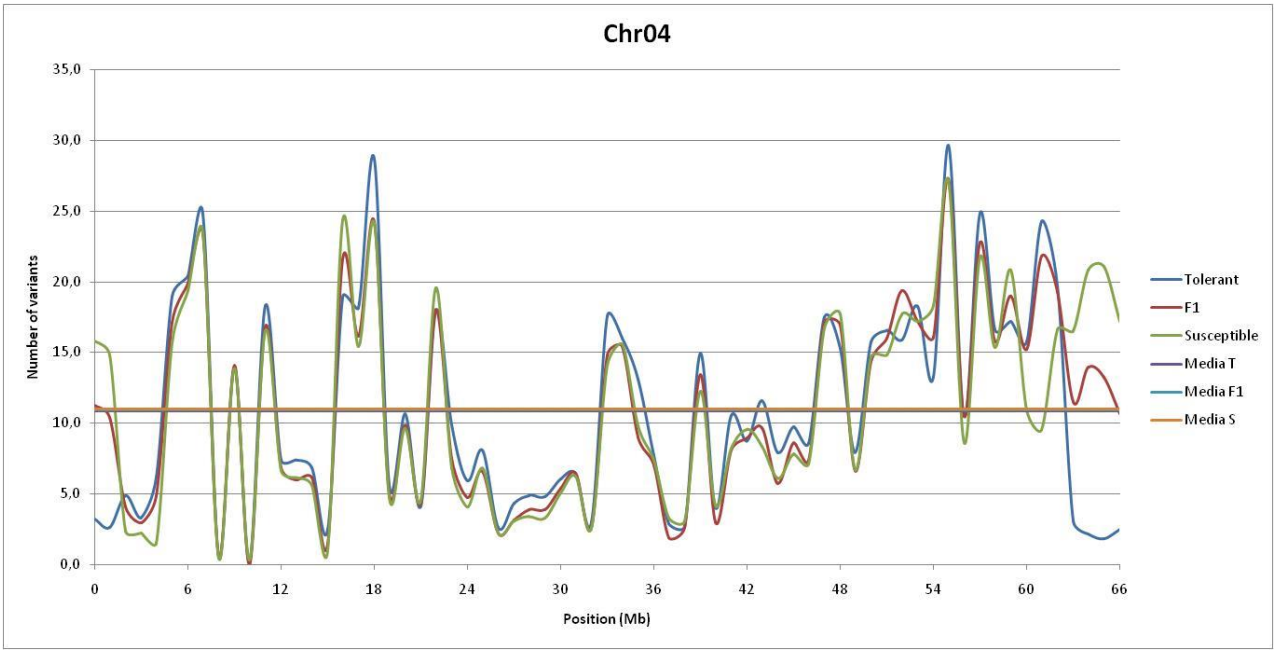
25
30
35
40
45
50
55
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S


Chr03
25,0
20,0
15,0
10,0
5,0
0,0
Number of variants
0
10
20
30
40
50
60
70
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S


Chr04
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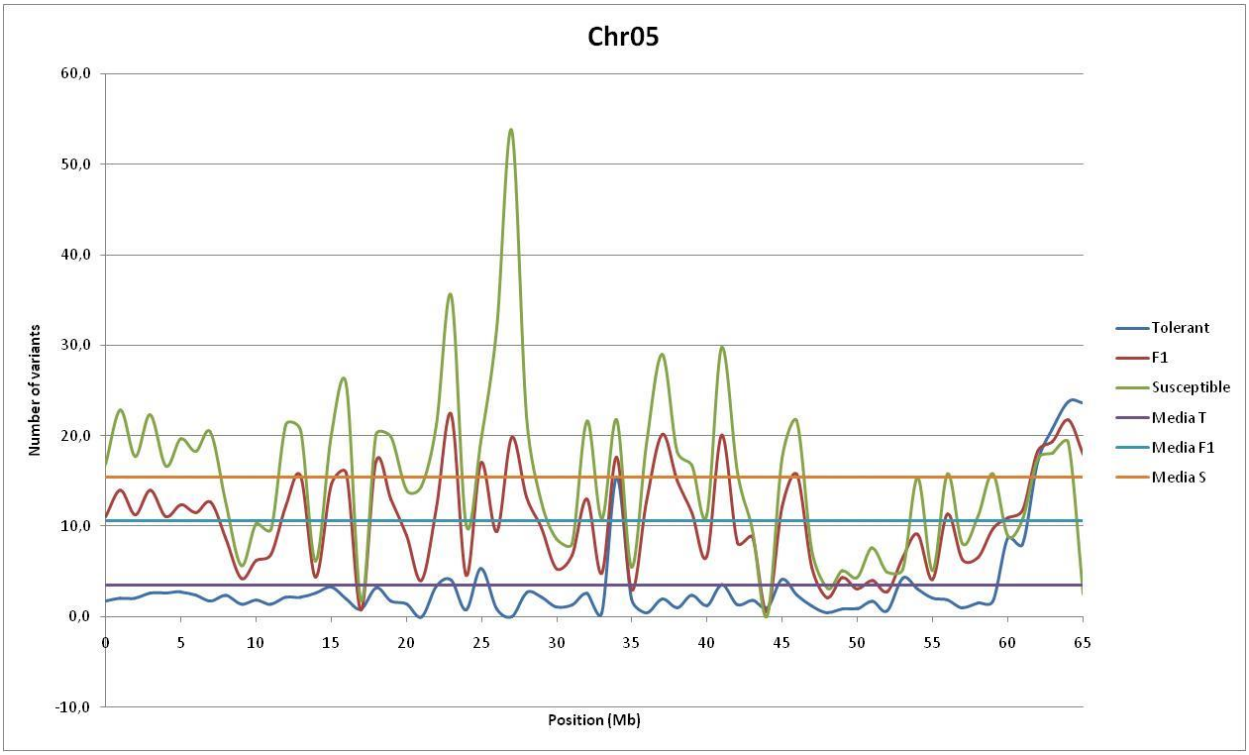
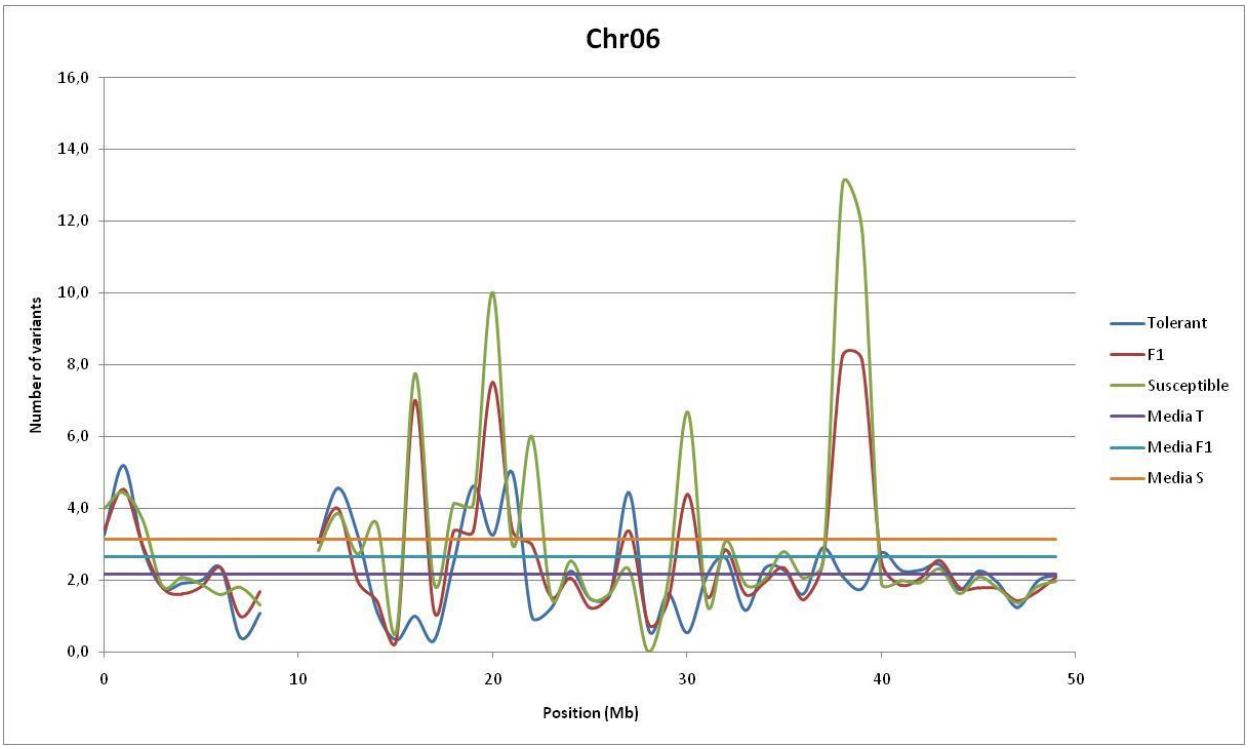
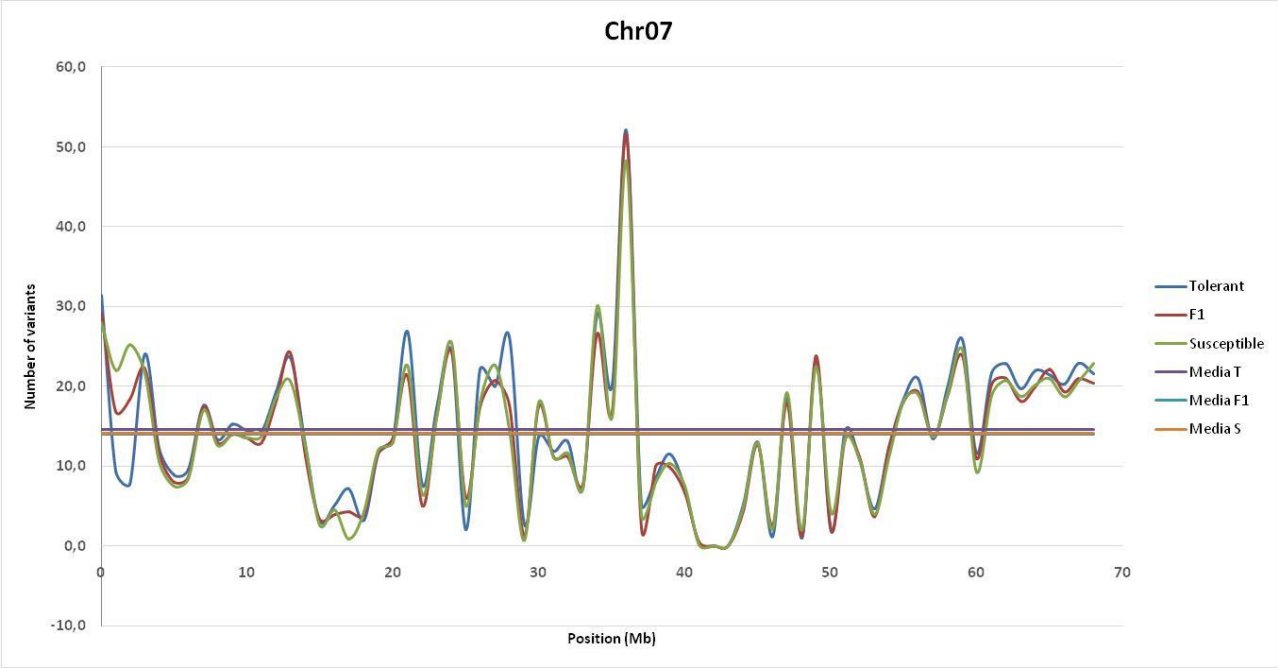
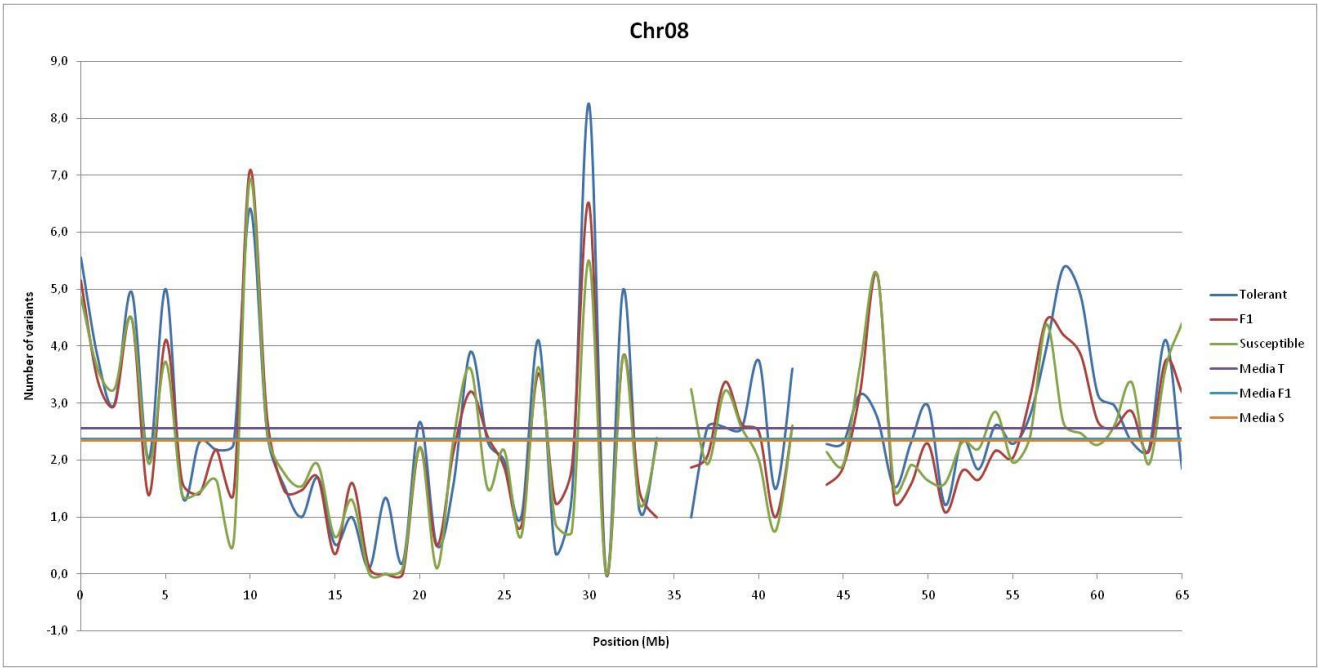
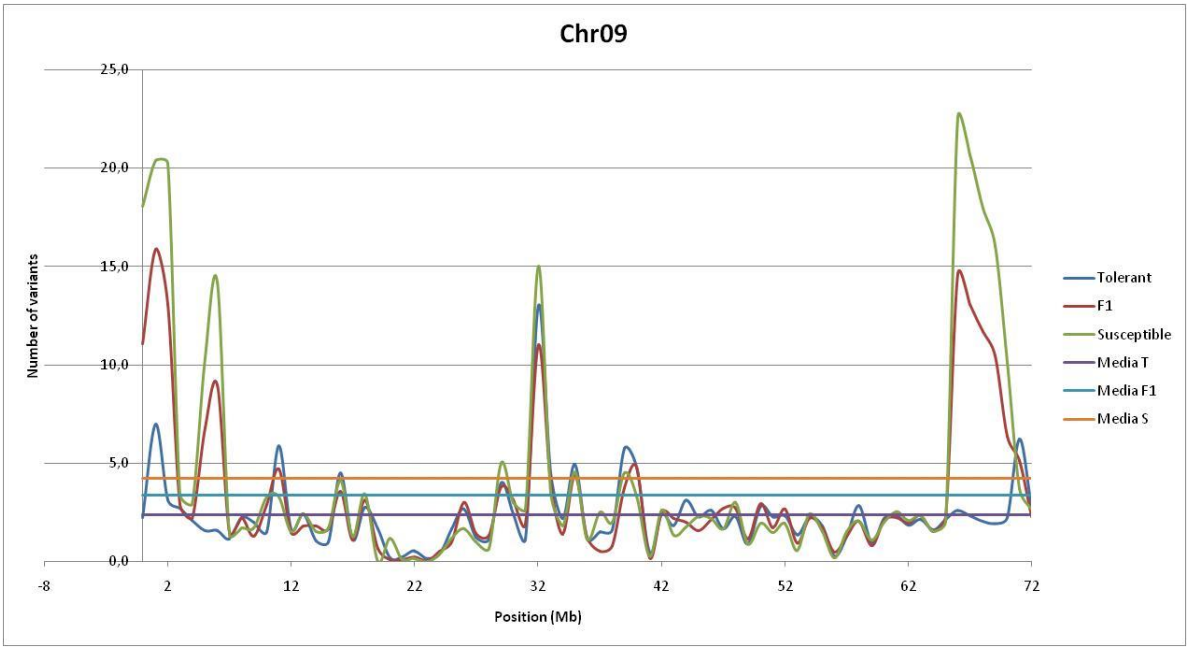
30,0
25,0
20,0
15,0
10,0
5,0
0,0
Number of variants
0
6
12
18
24
30
36
42
48
54
60
66
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S


Chr05
60,0
50,0
40,0
30,0
20,0
10,0
0,0
-10,0
Number of variants
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S


Chr06
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
Number of variants
0
10
20
30
40
50
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S


Chr07
60,0
50,0
40,0
30,0
20,0
10,0
0,0
-10,0
Number of variants
0
10
20
30
40
50
60
70
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S


Chr08
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
-1,0
Number of variants
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S


Chr09
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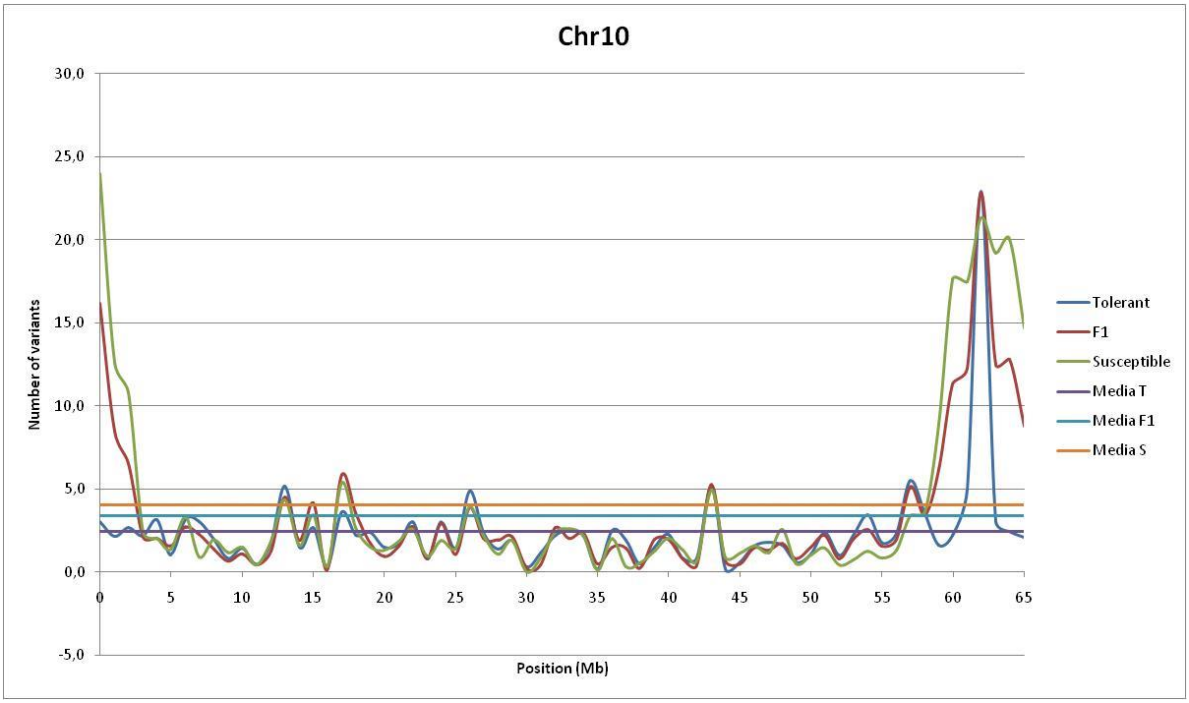
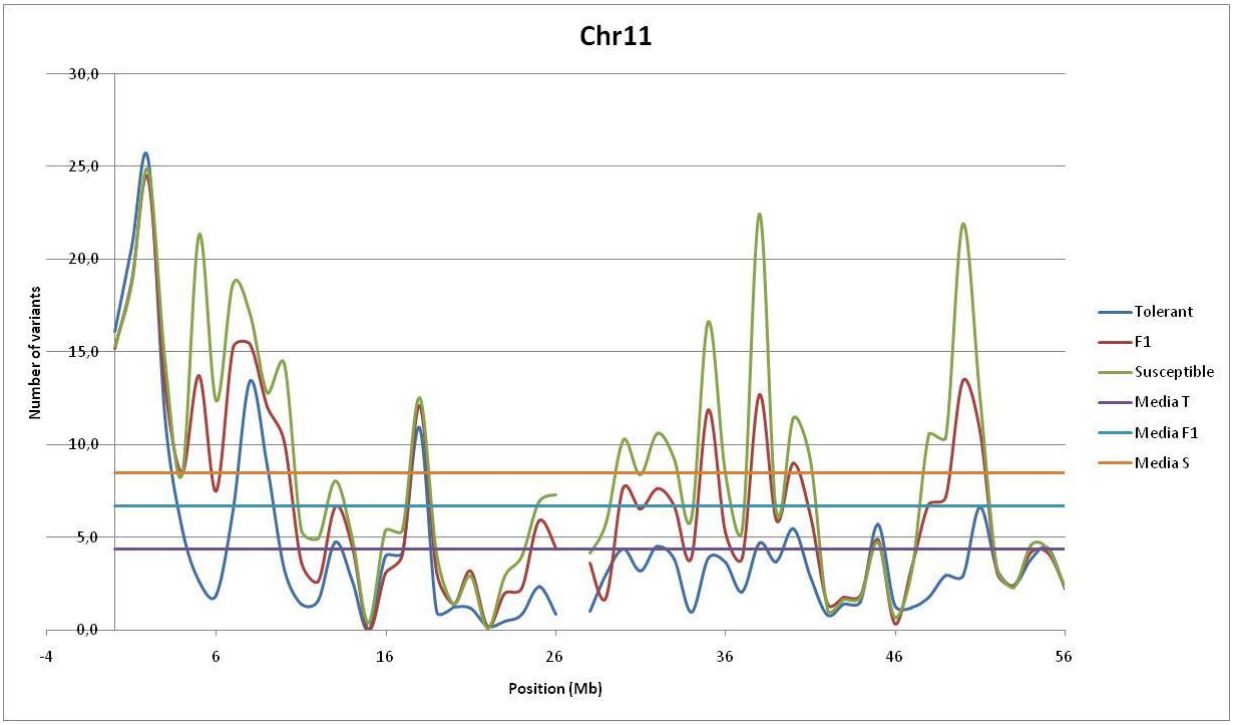
20,0
15,0
10,0
5,0
0,0
Number of variants
-8
2
12
22
32
42
52
62
72
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S


Chr10
30,0
25,0
20,0
15,0
10,0
5,0
0,0
-5,0
Number of variants
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Position (Mb)
Tolerant
F1
Susceptible
Media T
Media F1
Media S



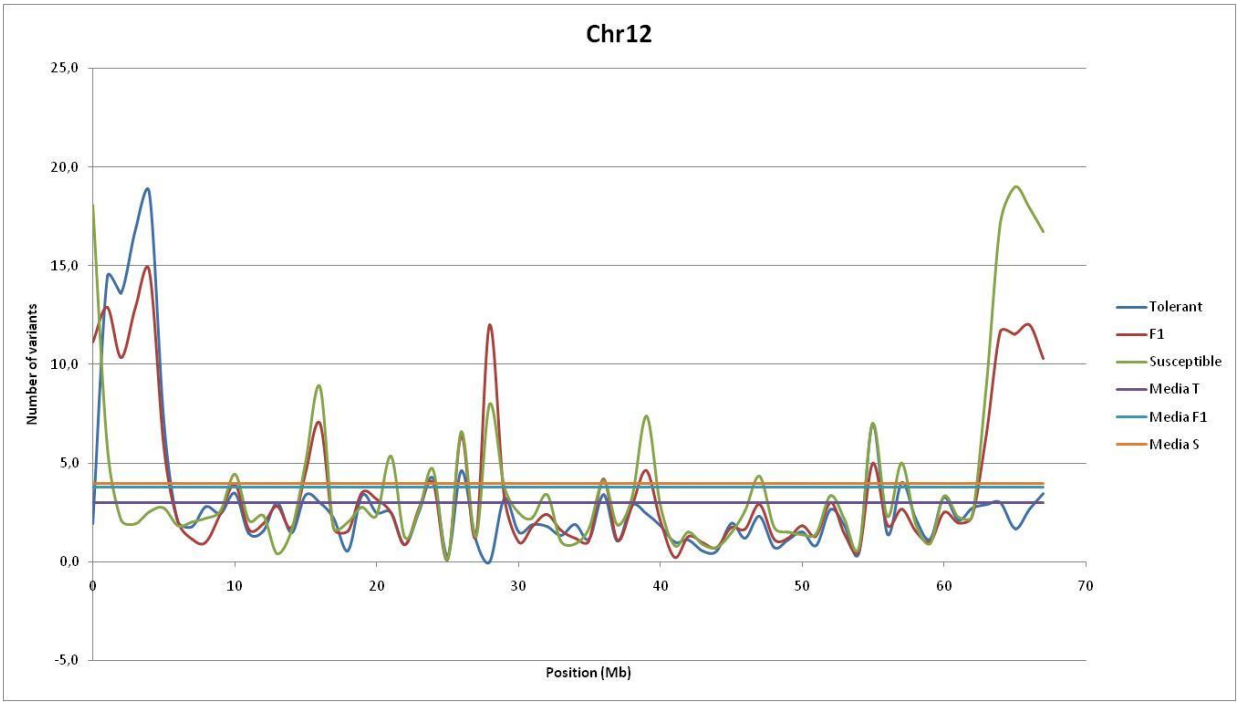


Figure S3. Figure showing the distribution variants along each chromosome over the three tomato genotypes (Tolerant, Susceptible and F1 hybrid), using a window size of 1MB. Bars referred to as 'Media T/S/F1' are the average number of variants in each chromosome, used as cut-off value to identify 'variant-peak' regions.

LIST OF PUBLICATIONS

Research article:

Manzo D, Ferriello F, Puopolo G, Zoina A, D'Esposito D, Tardella L, Ferrarini A, Ercolano MR. Fusarium oxysporumf.sp. radicis-lycopersici induces distinct transcriptome reprogramming in resistant and susceptible isogenic tomato lines.2016. *BMC Plant Biology***16**: 53.

Poster presentations:

WIKI-PRGDB: COMMUNITY-BASED PAGES ABOUT PLANT RESISTANCE GENES

D'Alessandro R., Sanseverino W., Hermoso Pulido A., Roma G., Lowy E., Vlasova A., Andolfo G., **Manzo D.**, Frusciante L.,Ercolano M.R.

VIPCA – Vienna International Plant Conference Association

Plant diseases and Resistence Mechanisms International Conference, Febbraio 2013

CAPS and HRM markers for rapid detection of ZYMV resistance loci in zucchini

Capuozzo C., **Manzo D.**, Formisano G., Ercolano M.R.

57th Annual Congress SIGA,

Foggia 16-19 September 2013

Transcriptional response of susceptible and tolerant tomato lines to Tuta absoluta

Manzo D., Borzi A., Garonna A., Rao R., Pennacchio F., Ercolano MR., Frusciante L.

58th Annual Congress SIGA,

Alghero 15-18 September 2014

Integrated genomic approaches for investigating the plant R genes machinery

Andolfo G., Capuozzo C., Di Donato A., Iovieno P., **Manzo D.**, Nieri D., Ercolano M. R.

XVI IS-MPMI 2014 - Molecular Plant-microbe Interactions 6-10 July - Rhodes, GREECE

Transcriptomic analysis of the three-way interaction between plant, pathogen and Trichoderma or its secondary metabolite

Manganiello G., **Manzo D.**, Sacco A., Vinale F., Pascale A., Ruocco M., Lanzuise S., Varlese R. , Marra R., Blad C., Innocente L., Lombardi N., Lorito M., Ercolano M., Woo S.L. 2014.

XX Convegno Nazionale SIPaV, Pisa, Italia, 22-24 Settembre 2014.

Integrated genomic approaches for investigating the plant R genes machinery

Andolfo A., Capuozzo C., Di Donato A., Iovieno P.,**Manzo D.**, Nieri D., Ercolano M.R.

The 18th Joint Meeting of EAPR Breeding and Varietal Assessment Section and EUCARPIA Section Potatoes, November 15 – 18, 2015, Vico Equense, Italy.